IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
BRAJE et al.

Serial No. 10/823,317

Filed: April 13, 2004

Group Art Unit: 1624

Examiner: Emily B. Bernhardt

For : N-[(Piperazinyl)hetaryl]arylsulfonamide compounds

DECLARATION

1. I, Wilfried M. Braje, Dr. rer. nat., a citizen of the Federal Republic of Germany and residing at Unter dem Hopfenberge 15, 31737 Rinteln, Germany, hereby declare as follows:

I am a fully trained Chemist having studied Chemistry at the University of Hannover, Germany, from 1990 to 1996, at the University of Hawaii, USA, from 01/1994 to 10/1994 and at Stanford University, USA from 06/1995 to 04/1996. I received a Diploma Degree in 01/1996 by the University of Hannover, Germany. In 1999, I received the doctorate degree (Ph.D.) by the University of Hannover, Germany.

I joined BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany, in 2000 and relocated to Abbott GmbH&Co. KG, 67061 Ludwigshafen, Germany, in 2001. Since then, I have been working in the field of medicinal chemistry. I have read and fully understood US application Ser. No. 10/823,317 and I am familiar with the subject-matter disclosed and claimed therein;

2. I have read and fully understood the Office Action of June 1, 2006 and the references cited therein by the Examiner;

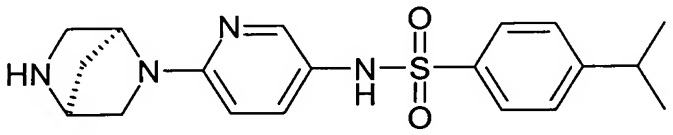
3. The following observations are made by me.

4. Supplementary Experimental Data

4.1 In order to provide further support for the compounds of formula I of claim 1, following additional synthesis examples, physicochemical and biological test data are presented.

4. 1.1 Synthesis examples

A) Synthesis of N-[(1S,4S)-6-(2,5-Diaza-bicyclo[2.2.1]hept-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide



A.1) (1S,4S)-2-Benzyl-5-(5-nitro-pyridin-2-yl)-2,5-diaza-bicyclo[2.2.1]heptane

To 2-chlor-5-nitropyridine (666 mg, 4.2 mmol), (1S,4S)-2-benzyl-2,5-diazabicyclo[2.2.1]heptane (1.47 g, 4.2 mmol), benzyltrimethylammonium chloride (37 mg, 0.2 mmol) and potassium carbonate (2.322 g, 16.8 mmol) was added dimethylformamide (DMF) (20 ml). The reaction mixture was stirred for 1 h at room temperature. Water (200 ml) was added and the mixture was extracted twice with ethyl acetate (100 ml). The combined organic phases were washed with water and subsequently dried with sodium sulfate, filtered and concentrated in vacuo to give the desired crystalline product (1.21 g, 93 % yield).

MS [m+1]: 311.15

A.2) 6-((1S,4S)-5-Benzyl-2,5-diaza-bicyclo[2.2.1]hept-2-yl)pyridin-3-ylamine

(1S,4S)-2-Benzyl-5-(5-nitro-pyridin-2-yl)-2,5-diaza-bicyclo[2.2.1]heptane (1.2 g, 3.87 mmol) was dissolved in methanol (40 ml). Stannous dichloride (7.85 g, 34.8 mmol) was added, and the reaction mixture was stirred overnight at room temperature. Methanol was removed, the residue was treated with 1 N

aqueous sodium hydroxide to reach pH 9 and dichloromethane (50 ml) was added. The precipitated solid was filtered off and the aqueous phase was extracted twice with dichloromethane (100 ml). The combined organic phases were dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure to give the desired product (880 mg, 76 % yield).

MS [m+1]: 281.15

A.3) N-[6-((1S,4S)-5-Benzyl-2,5-diaza-bicyclo[2.2.1]hept-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide

6-((1S,4S)-5-Benzyl-2,5-diaza-bicyclo[2.2.1]hept-2-yl)pyridin-3-ylamine (880 mg, 2.95 mmol), 4-isopropyl-benzene sulfonylchloride (582 µl, 3.25 mmol) and triethyl amine (1.23 ml, 8.86 mmol) were dissolved in THF (30 ml). The reaction mixture was stirred for 1 h at room temperature. The solvent was removed and water (100 ml) was added. The aqueous phase was extracted twice with diethyl ether. The organic phases were combined, dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure to yield the product (1.14 g, 83 % yield).

MS [m+1]: 463.25

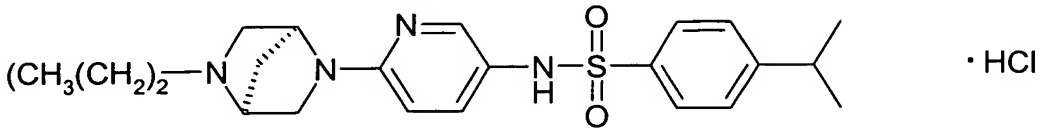
A.4) N-[(1S,4S)-6-(2,5-Diaza-bicyclo[2.2.1]hept-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide

A mixture of N-[6-((1S,4S)-5-Benzyl-2,5-diaza-bicyclo[2.2.1]hept-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide (1.14 g, 2.46 mmol) and 10 % palladium on carbon (50 mg) in a mixture of ethyl acetate (50 ml) and acetic acid (20 ml) was hydrogenated overnight. The catalyst was filtered off, and the solvent was removed under vacuum. The residue was dissolved in distilled H_2O (50 ml) and extracted three times with ethyl acetate (150 ml). The organic phases were combined, dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure to yield the title compound (640 mg, 70 % yield).

MS [m+1]: 373.15

^{1}H-NMR (d_6-DMSO): δ = 7.65 (d, 1H); 7.6 (d, 2H); 7.4 (d, 2H); 7.15 (dd, 1H); 6.85 (d, 1H); 4.55 (s, 1H); 3.6 (s, 1H); 3.35 (dd, 1H); 3.05 (d, 1H); 2.95 (sept. 1H); 2.85 (d, 1H); 2.7 (d, 1H); 1.7 (d, 1H); 1.6 (d, 1H); 1.2 (d, 6H).

B) 4-Isopropyl-N-[6-((1S,4S)-5-propyl-2,5-diaza-bicyclo[2.2.1]hept-2-yl)-pyridin-3-yl]-benzenesulfonamide, hydrochloride

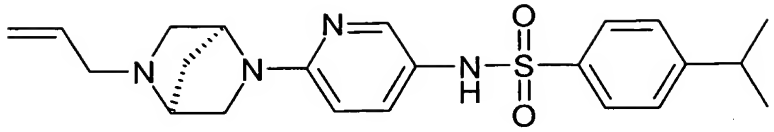


N-[(1S,4S)-6-(2,5-Diaza-bicyclo[2.2.1]hept-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide (300 mg, 0.81 mmol) and propionaldehyde (88 µl, 1.21 mmol) were dissolved in THF (20 ml). Acetic acid (63 µl, 1.21 mmol) and sodium trisacetoxyborohydride (341 mg, 1.21 mmol) were sequentially added to the reaction mixture and stirred for 30 minutes at room temperature. The reaction mixture was concentrated and the residue was dissolved in H_2O (50 ml) and twice extracted with ethyl acetate (50 ml). The organic phases were combined, dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure. The residue was dissolved in diethyl ether (25 ml) and HCl in diethyl ether solution was added. The precipitate was collected to yield the desired product (235 mg, 62 % yield).

MS [m+1]: 415.25

H-NMR (d_6-DMSO): δ = 10.35 (bs, 1H); 10.05 (bs, 1H); 7.7 (s, 1H); 7.65 (d, 2H); 7.45 (d, 2H); 7.4 (s, 1H); 6.75 (m, 1H); 4.9 (s, 1H); 4.5 (s, 1H); 3.9 (d, 1H); 3.75 (d, 1H); 3.6 (d, 1H); 3.55 (m, 1H); 3.2 (m, 1H); 3.0 (m, 2H); 2.35 (d, 1H); 2.1 (d, 1H); 1.7 (m, 2H); 1.2 (d, 6H); 0.9 (t, 3H).

C) N-[6-((1S,4S)-5-Allyl-2,5-diaza-bicyclo[2.2.1]hept-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide, hydrochloride



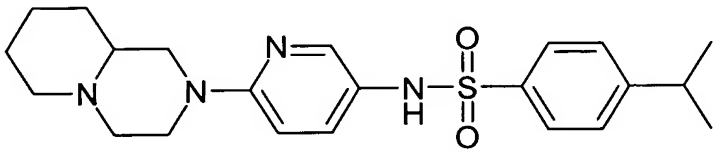
· HCl

N-[(1S,4S)-6-(2,5-Diaza-bicyclo[2.2.1]hept-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide (300 mg, 0.81 mmol) was dissolved in DMF (10 ml). Allyl bromide (105 µl, 1.21 mmol) and triethyl amine (0.45 ml, 3.22 mmol) were added and the solution was stirred for 1 h at room temperature. Water (90 ml) was added and extracted twice with ethyl acetate (50 ml). The combined organic phases were washed with water (25 ml), and subsequently dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was dissolved in diethyl ether (25 ml) and HCl in diethyl ether solution was added. The precipitate was collected to yield the desired product (272 mg, 70 % yield).

MS [m+1]: 413.25

^{1}H-NMR (d_6-DMSO): δ = 10.8 (bs, 1H); 10.05 (bs, 1H); 7.7 (d, 1H); 7.65 (d, 2H); 7.45 (d, 2H); 7.4 (d, 1H); 6.75 (m, 1H); 5.95 (m, 1H); 5.5 (m, 2H); 4.9 (s, 1H); 4.45 (s, 1H); 3.95 (m, 1H); 3.9 (d, 1H); 3.75 (m, 1H); 3.6 (d, 1H); 3.45 (m, 1H); 3.15 (d, 1H); 2.95 (m, 2H); 2.4 (d, 1H); 2.1 (d, 1H); 1.2 (d, 6H)..

D) 4-Isopropyl-N-[6-(octahydro-pyrido[1,2-a]pyrazin-2-yl)-pyridin-3-yl]-benzenesulfonamide



D.1) 2-(5-Nitro-pyridin-2-yl)-octahydro-pyrido[1,2-a]pyrazine

2-Chlor-5-nitropyridine (1.13 g, 7.13 mmol) and potassium carbonate (1.97 g, 14.26 mmol) were dissolved in DMF (10 ml) and stirred for 30 minutes at 0 °C. 1,4-Diazabicyclo[4.4.0]decane was added and the reaction was stirred at room

temperature overnight. The reaction mixture was concentrated and the residue was dissolved in H_2O (50 ml) and twice extracted with diethyl ether (50 ml). The combined organic phases were washed twice with water and subsequently dried with sodium sulfate, filtered and concentrated in vacuum to give the desired product (1.87 g, 100 % yield).

MS [m+1]: 263.15

D.2) 6-(Octahydro-pyrido[1,2-a]pyrazin-2-yl)-pyridin-3-ylamine

2-(5-Nitro-pyridin-2-yl)-octahydro-pyrido[1,2-a]pyrazine (1.87 g, 7.12 mmol) was dissolved in methanol (50 ml), stannous dichloride was added (14.46 g, 64.1 mmol), and the reaction mixture was stirred at reflux for 3 h. Methanol was removed, and the residue was treated with 1 N aqueous sodium hydroxide to reach pH 9. Ethyl acetate was added and the precipitated solid was filtered off. The aqueous phase was extracted three times with dichloromethane. The combined organic phases were dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure to give the desired product (1.42 g, 86 % yield).

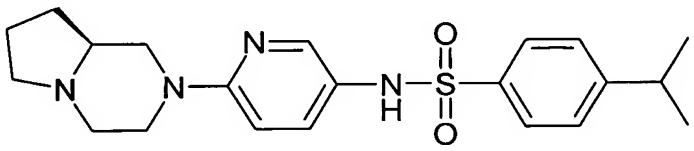
D.3) 4-Isopropyl-N-[6-(octahydro-pyrido[1,2-a]pyrazin-2-yl)-pyridin-3-yl]-benzenesulfonamide

6-(Octahydro-pyrido[1,2-a]pyrazin-2-yl)-pyridin-3-ylamine (300 mg, 1.29 mmol, 4-isopropyl-benzene sulfonylchloride (243 µl. 1.36 mmol) and triethyl amine (0.54 ml, 3.87 mmol) were dissolved in 10 ml THF. The reaction mixture was stirred overnight at room temperature. The solvent was removed and 100 ml water was added. The aqueous phase was extracted twice with diethyl ether. The organic phases were combined, dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel chromatography with cyclohexane/ethyl acetate (60-80%) as eluent, yielding the purified product (385 mg, 72 %).

MS [m+1]: 415.15

^{1}H-NMR (d_6-DMSO): δ = 7.7 (s, 1H); 7.65 (d, 2H); 7.4 (dd, 2H); 7.35 (d, 1H); 6.55 (d, 1H); 4.05 (m, 2H); 3.05-2.8 (m, 4H); 2.6 (t, 1H); 2.25 (m, 1H); 2.05 (m, 1H); 1.95 (m, 1H); 1.8 (s, 1H); 1.65 (m, 4H); 1.3 (m, 7H).

E) N-[(S)-6-(Hexahydro-pyrrolo[1,2-a]pyrazin-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide, hydrochloride



· HCl

E.1) (S)-2-(5-Nitro-pyridin-2-yl)-octahydro-pyrrolo[1,2-a]pyrazine

2-Chlor-5-nitropyridine (1.256 g, 7.92 mmol) and potassium carbonate (2.19 g, 15.85 mmol) were dissolved in DMF (10 ml) and stirred for 30 minutes at 0 °C. (S)-1,4-Diazabicyclo[4.3.0]nonane was added and the reaction was stirred at room temperature overnight. The reaction mixture was concentrated and the residue was dissolved in H_2O (50 ml) and twice extracted with diethyl ether (50 ml). The combined organic phases were washed twice with water and subsequently dried with sodium sulfate, filtered and concentrated in vacuum to give the desired product (1.84 g, 94 % yield).

MS [m+1]: 249.15

E.2) (S)-6-(Hexahydro-pyrrolo[1,2-a]pyrazin-2-yl)-pyridin-3-ylamine

(S)-2-(5-Nitro-pyridin-2-yl)-octahydro-pyrrolo[1,2-a]pyrazine (1.84 g, 7.41 mmol) was dissolved in methanol (50 ml), stannous dichloride was added (15.05 g, 66.7 mmol), and the reaction mixture stirred at reflux for 3 h. Methanol was removed, and the residue was treated with 1 N aqueous sodium hydroxide to reach pH 9. Ethyl acetate was added and the precipitated solid was filtered off. The aqueous phase was extracted three times with dichloromethane. The combined organic phases were dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure to give the desired product (1.36 g, 84 % yield).

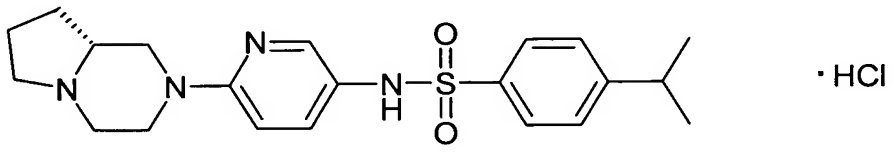
E.3) N-[(S)-6-(Hexahydro-pyrrolo[1,2-a]pyrazin-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide, hydrochloride

(S)-6-(Hexahydro-pyrrolo[1,2-a]pyrazin-2-yl)-pyridin-3-ylamine (400 mg, 1.83 mmol), 4-isopropyl-benzene sulfonylchloride (345 µl, 1.92 mmol) and triethyl amine (0.77 ml, 5.5 mmol) were dissolved in 10 ml THF. The reaction mixture was stirred overnight at room temperature. The solvent was removed and 100 ml of water were added. The aqueous phase was extracted twice with diethyl ether. The organic phases were combined, dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel chromatography with cyclohexane/ethyl acetate (30 %) and ethyl acetate/methanol (5 %) as eluent. The residue was dissolved in dichloromethane (5 ml) and HCl in diethyl ether solution was added. The precipitate was collected to yield the desired product (30 mg, 4 % yield).

MS [m+1]: 401.25

^{1}H-NMR (d_6-DMSO): δ = 11.3/11.05 (bs, 1H); 9.95 (s, 1H); 7.8/7.75 (s, 1H); 7.65 (d, 2H); 7.45 (d, 2H); 7.35 (m, 1H); 6.9/6.85 (d, 1H); 4.6/4.35 (d, 1H); 3.95 (m, 1H); 3.8-3.65 (m, 2H); 3.6-2.95 (m, 5H); 2.2-1.85 (m, 3H); 1.75 (m, 1H); 1.2 (m, 7H).

F) N-[(R)-6-(Hexahydro-pyrrolo[1,2-a]pyrazin-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide, hydrochloride



F.1) (R)-2-(5-Nitro-pyridin-2-yl)-octahydro-pyrrolo[1,2-a]pyrazine

2-Chlor-5-nitropyridine (600 mg, 4.75 mmol) and potassium carbonate (1.314 g, 9.51 mmol) were dissolved in DMF (10 ml) and stirred for 30 minutes at

0°C. (R)-1,4-Diazabicyclo[4.3.0]nonane was added and the reaction was stirred for 3 h at room temperature. The reaction mixture was concentrated and the residue was dissolved in H_2O (50 ml) and three times extracted with ethyl acetate (50 ml). The combined organic phases were washed twice with water and subsequently dried with sodium sulfate, filtered and concentrated in vacuum to give the desired product (1.1 g, 93 % yield).

MS [m+1]: 249.15

F.2) (R)-6-(Hexahydro-pyrrolo[1,2-a]pyrazin-2-yl)-pyridin-3-ylamine

(R)-2-(5-Nitro-pyridin-2-yl)-octahydro-pyrrolo[1,2-a]pyrazine (1.1 g, 4.43 mmol) was dissolved in methanol (50 ml), stannous dichloride was added (9 g, 39.87 mmol), and the reaction mixture was stirred at reflux for 3 h. Methanol was removed, and the residue was treated with 1 N aqueous sodium hydroxide to reach pH 9. Ethyl acetate was added and the precipitated solid was filtered off. The aqueous phase was extracted three times with dichloromethane. The combined organic phases were dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure to give the desired product (1.12 g, 80 % purity, 93 % yield).

MS [m+1]: 219.15

F.3) N-[(R)-6-(Hexahydro-pyrrolo[1,2-a]pyrazin-2-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide, hydrochloride

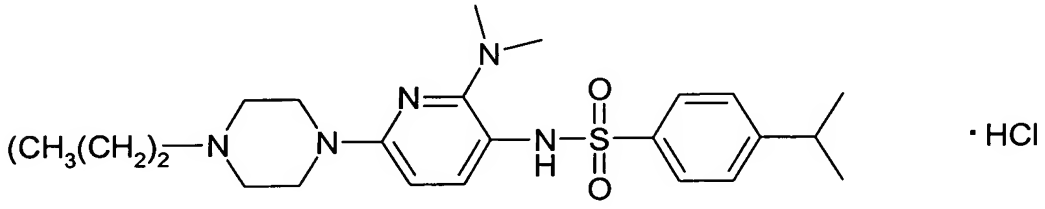
(R)-6-(Hexahydro-pyrrolo[1,2-a]pyrazin-2-yl)-pyridin-3-ylamine (400 mg, 80 % purity, 1.47 mmol), 4-isopropyl-benzene sulfonylchloride (263 µl. 1.47 mmol) and triethyl amine (0.61 ml, 4.4 mmol) were dissolved in 10 ml THF. The reaction mixture was stirred overnight at room temperature. The solvent was removed and 100 ml of water were added. The aqueous phase was extracted twice with diethyl ether. The organic phases were combined, dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel chromatography with ethyl acetate/methanol (5 %) as eluent. The residue was dissolved in

dichloromethane (5 ml) and HCl in diethyl ether solution was added. The precipitate was collected to yield the desired product (150 mg, 23 % yield).

MS [m+1]: 401.25

^{1}H-NMR (d_6-DMSO): δ = 11.3/11.05 (bs, 1H); 9.95 (s, 1H); 7.8/7.75 (s, 1H); 7.65 (d, 2H); 7.45 (d, 2H); 7.35 (d, 1H); 6.9/6.8 (d, 1H); 4.6/4.35 (d, 1H); 3.8-2.95 (m, 8H); 2.2-1.85 (m, 3H); 1.75 (m, 1H); 1.2 (m, 7H).

G) N-[2-Dimethylamino-6-(4-propyl-piperazin-1-yl)-pyridin-3-yl]-4-isopropyl-benzensulfonamide, hydrochloride



G.1) 1-Benzyl-4-(6-chloro-5-nitro-pyridin-2-yl)-piperazine and 1-Benzyl-4-(6-chloro-3-nitro-pyridin-2-yl)-piperazine

2,6-Dichlor-3-nitropyridine (1.0 g, 4.77 mmol) was dissolved in DMF (50 ml), benzylpiperazine (840 mg, 4.77 mmol) was added and the reaction was stirred overnight at room temperature. To the reaction mixture was added water (250 ml) and NaOH solution. The aqueous phase was extracted twice with ethyl acetate. The organic phases were combined, dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel chromatography with cyclohexane/ethyl acetate (5%-15%) as eluent to yield 1-benzyl-4-(6-chloro-5-nitro-pyridin-2-yl)-piperazine (200 mg, 13 % yield) and of 1-benzyl-4-(6-chloro-3-nitro-pyridin-2-yl)-piperazine (900 mg, 57 % yield).

1-Benzyl-4-(6-chloro-5-nitro-pyridin-2-yl)-piperazine

MS [m+1]: 333.05

^{1}H-NMR (d_6-DMSO): δ [ppm] 8.3 (d, 1H); 7.4-7.25 (m, 5H); 6.95 (d, 1H); 3.75 (bs, 4H); 3.55 (s, 2H); 2.5 (m, 4H).

1-Benzyl-4-(6-chloro-3-nitro-pyridin-2-yl)-piperazine

MS [m+1]: 333.05

^{1}H-NMR (d_6-DMSO): δ [ppm] 8.3 (d, 1H); 7.35 (m, 4H); 7.3 (m, 1H); 6.9 (d, 1H); 3.55 (s, 2H); 3.4 (bs, 4H); 2.5 (m, 4H).

G.2) [6-(4-Benzyl-piperazin-1-yl)-3-nitro-pyridin-2-yl]-dimethyl-amine

1-Benzyl-4-(6-chloro-5-nitro-pyridin-2-yl)-piperazine (200 mg, 0.60 mmol) was dissolved in THF (10 ml), a 2 molar solution of dimethylamine in THF (750 µl, 1.5 mmol) was added and the reaction was stirred overnight at room temperature. The solvent was removed and water (50 ml) was added. The aqueous phase was extracted twice with ethyl acetate. The organic phases were combined, dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure to yield the desired product (220 mg).

MS [m+1]: 342.15

G.3) 6-(4-Benzyl-piperazin-1-yl)-N*2*,N*2*-dimethyl-pyridine-2,3-diamine

[6-(4-Benzyl-piperazin-1-yl)-3-nitro-pyridin-2-yl]-dimethyl-amine (220 mg, 0.64 mmol) was dissolved in methanol (50 ml), stannous dichloride was added (1.31 g, 5.80 mmol), and the reaction mixture was stirred at reflux for 17 h. Methanol was removed, and the residue was treated with 1 N aqueous sodium hydroxide to reach pH 9. Ethyl acetate was added and the precipitating solid was filtered off. The aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure to give the desired product (210 mg, 95 % purity, 99 % yield).

MS [m+1]: 312.25

G.4) N-[6-(4-Benzyl-piperazin-1-yl)-2-dimethylamino-pyridin-3-yl]-4-isopropyl-benzensulfonamide

6-(4-Benzyl-piperazin-1-yl)-N*2*,N*2*-dimethyl-pyridine-2,3-diamine (210 mg, 95 % purity, 0.64 mmol), 4-isopropyl-benzene sulfonylchloride (115 µl. 0.64 mmol) and triethyl amine (0.27 ml, 1.92 mmol) were dissolved in 25 ml THF. The reaction mixture was stirred for 7 h at 50°C. 4-Isopropyl-benzene sulfonylchloride (33 µl. 0.19 mmol) was added and the reaction mixture was stirred at room temperature overnight. The solvent was removed and aqueous NaOH solution was added. The aqueous phase was extracted twice with ethyl acetate. The combined organic phases were extracted once with 1 N HCl solution. The acidic aqueous phases was made alkaline with NaOH and then extracted twice with ethyl acetate. These two organic phases were combined, dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure to give the crude product (130 mg, 80 % purity, 33 % yield).

MS [m+1]: 494.25

G.5) N-(2-Dimethylamino-6-piperazin-1-yl-pyridin-3-yl)-4-isopropyl-benzensulfonamide

A mixture of N-[6-(4-Benzyl-piperazin-1-yl)-2-dimethylamino-pyridin-3-yl]-4-isopropyl-benzensulfonamide (130 mg, 0.21 mmol) and 10 % palladium on carbon (10 mg) in a mixture of ethyl acetate (20 ml) and acetic acid (5 ml) was hydrogenated overnight. Further quantities of 10 % palladium on carbon and acetic acid (2 ml) were added. The catalyst was filtered off, and the solvent was removed under vacuum. The residue was treated with aqueous 1 N NaOH solution and extracted twice with ethyl acetate (100 ml). The organic phases were combined, dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure to yield the product. The crude product was purified by silica gel chromatography to give the desired product (23 mg, 27 % yield).

MS [m+1]: 404.15

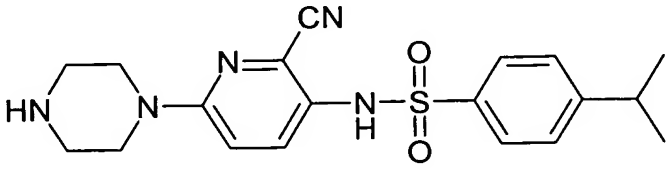
G.6) N-[2-Dimethylamino-6-(4-propyl-piperazin-1-yl)-pyridin-3-yl]-4-isopropyl-benzensulfonamide, hydrochloride

N-(2-Dimethylamino-6-piperazin-1-yl-pyridin-3-yl)-4-isopropyl-benzensulfonamide (23 mg, 0.06 mmol) and propionaldehyde (4 µl, 0.06 mmol) were dissolved in THF (5 ml). Acetic acid (5 µl, 0.09 mmol) and sodium trisacetoxyborohydride (18 mg, 0.09 mmol) were sequentially added to the reaction mixture and stirred for 1 h at room temperature. The reaction mixture was concentrated and the residue was dissolved in aqueous $NaHCO_3$ solution and extracted with diethyl ether. The organic phases were dried over magnesium sulfate, filtered, and the solvent was evaporated under reduced pressure. The residue was dissolved in diethyl ether (25 ml) and HCl in diethyl ether solution was added. The precipitate was collected to yield the desired product (19 mg, 69 % yield).

MS [m+1]: 446.25

^{1}H-NMR (d_6-DMSO): δ [ppm] 10.8 (bs, 1H); 9.2 (bs, 1H); 7.6 (d, 2H); 7.45 (d, 2H); 6.7 (d, 1H); 6.15 (bs, 1H); 4.25 (d, 2H); 3.5 (d, 2H); 3.25 (t, 2H); 3.0 (m, 5H); 2.9 (s, 6H); 1.75 (m, 2H); 1.2 (d, 6H); 0.9 (t, 3H).

H) N-(2-Cyano-6-piperazin-1-yl-pyridin-3-yl)-4-isopropyl-benzenesulfonamide



H.1) 4-(6-Cyano-5-nitro-pyridin-2-yl)-piperazine-1-carboxylic acid tert-butyl ester

The compound was prepared from piperazine-1-carboxylic acid tert-butyl ester and 6-chloro-3-nitro-pyridine-2-carbonitrile by the method described for Example 1 of the present application . Yield: 6.9 g (77%).

ESI-MS: 234.5 $[M+H - Boc]^+$, 334.2 $[M+H]^+$

^{1}H-NMR (DMSO, 400 MHz): δ [ppm] 1.47 (s, 9H), 3.57 (m, 4H), 3.80 (m, 4H), 6.77 (d, 1H), 8.30 (d, 1H).

H.2) 4-(5-Amino-6-cyano-pyridin-2-yl)-piperazine-1-carboxylic acid tert-butyl ester

The compound was prepared by reduction of 4-(6-cyano-5-nitro-pyridin-2-yl)-piperazine-1-carboxylic acid tert-butyl ester by the method described for Example 1 of the present application. Yield: 5.60 g (90%).

ESI-MS: 204.1 $[M+H - Boc]^+$, 304.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$, 400 MHz): δ [ppm] 1.47 (s, 9H), 3.32 (m, 4H), 3.50 (m, 4H), 3.93 (s, 2H), 6.81 (d, 1H), 7.02 (d, 1H).

H.3) 4-[6-Cyano-5-(4-isopropyl-benzenesulfonylamino)-pyridin-2-yl]-piperazine-1-carboxylic acid tert-butyl ester

The compound was prepared from 4-(5-amino-6-cyano-pyridin-2-yl)-piperazine-1-carboxylic acid tert-butyl ester and 4-isopropyl-benzenesulfonyl chloride by the method described for Example 1 of the present application. Yield: 0.26 g (81%).

MS (ESI) m/z: 430.2 $[M+H - tBu]^+$

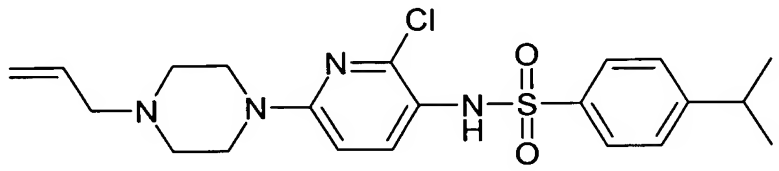
H.4) N-(2-Cyano-6-piperazin-1-yl-pyridin-3-yl)-4-isopropyl-benzenesulfonamide

The compound was prepared by acidic deprotection of 4-[6-cyano-5-(4-isopropyl-benzenesulfonylamino)-pyridin-2-yl]-piperazine-1-carboxylic acid tert-butyl ester by the method described for Example 1 of the present application. Yield: 0.12 g (88%).

ESI-MS: 386.1 $[M+H]^+$

^{1}H-NMR ($CDCl_3$, 400 MHz): δ [ppm] 1.21 (d, 6H), 2.98 (m, 1H), 3.14 (m, 4H), 3.70 (m, 4H), 7.17 (m, 2H), 7.46 (d, 2H), 7.52 (d, 2H), 9.00 (br s, 1H), 10.20 (br s, 1H).

l) N-[6-(4-Allyl-piperazin-1-yl)-2-chloro-pyridin-3-yl]-4-isopropyl-benzenesulfonamide



l.1) 1-Allyl-4-(6-chloro-5-nitro-pyridin-2-yl)-piperazine

The compound was prepared from piperazine-1-carboxylic acid tert-butyl ester and 2,6-dichloro-3-nitro-pyridine by the method described for Example 1 of the present application. Yield: 11.0 g (93%).

ESI-MS: 243.1 $[M+H - Boc]^+$, 287.0 / 289.0 $[M+H - tBu]^+$

^{1}H-NMR (DMSO, 400 MHz): δ [ppm] 1.42 (s, 9H), 3.37 (m, 4H), 3.46 (m, 4H), 6.92 (d, 1H), 8.30 (d, 1H).

l.2) 6-(4-Allyl-piperazin-1-yl)-2-chloro-pyridin-3-ylamine

The compound was prepared by reduction of 1-allyl-4-(6-chloro-5-nitro-pyridin-2-yl)-piperazine by the method described for Example 1 of the present application. Yield: 2.22 g (94%)

ESI-MS: 253.1 $[M+H]^+$

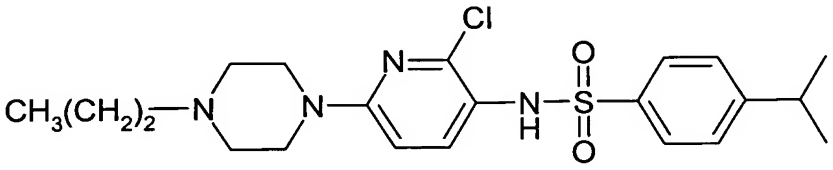
l.3) N-[6-(4-Allyl-piperazin-1-yl)-2-chloro-pyridin-3-yl]-4-isopropyl-benzenesulfonamide

The compound was prepared from 6-(4-allyl-piperazin-1-yl)-2-chloro-pyridin-3-ylamine and 4-isopropyl-benzenesulfonyl chloride by the method described for Example 1 of the present application. Yield: 1.96 g (65%).

ESI-MS: 435.1 $[M+H]^+$

^{1}H-NMR (DMSO): δ [ppm] 1.20 (m, 6H), 2.92 (m, 3H), 3.25 (m, 4H), 3.57 (m, 2H), 3.73 (m, 2H), 5.51 (m, 2H), 6.02 (m, 1H), 7.00 (d, 1H), 7.32 (d, 1H), 7.43 (d, 2H), 7.66 (d, 2H), 9.75 (br s, 1H).

J) N-[2-Chloro-6-(4-propyl-piperazin-1-yl)-pyridin-3-yl]-4-isopropyl-benzenesulfonamide

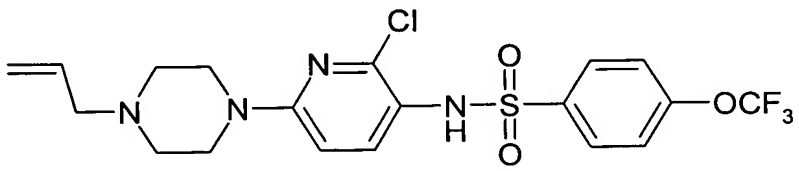


The compound was prepared by reduction of N-[6-(4-allyl-piperazin-1-yl)-2-chloro-pyridin-3-yl]-4-isopropyl-benzenesulfonamide by the method described for Example 17 of the present application. Yield: 0.20 g (46%).

ESI-MS: 437.1 $[M+H]^+$

According to the above procedures, the following compounds were synthesized.

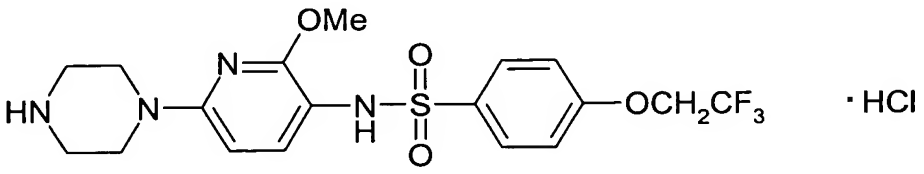
K) N-[2-Chloro-6-(4-allyl-piperazin-1-yl)-pyridin-3-yl]-4-trifluoromethoxy-benzenesulfonamide, dihydrochloride



· 2 HCl

MS of the dihydrochloride: 549.8

L) N-[2-Methoxy-6-(piperazin-1-yl)-pyridin-3-yl]-4-(2,2,2-trifluoroethoxy)-benzenesulfonamide, hydrochloride



MS: 482.9

4.1.2 Biological investigations

The receptor binding studies were carried out according to the method described in the present invention. The results are given in following table.

Example	K_i (D_3) [nM]	K_i (D_2) [nM]	$K_i(D_2)$ / $K_i(D_3)$
A	82.3		
B	16.9	517	31
C	11.1	191	17
D	11.2		
E	11.8	272	23
G	2.0	29.3	15
H	16	702	44
K	1.2		
L	3	298	99

As can be seen, the compounds have a good affinity and selectivity for the D_3 receptor.

5. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1101 of Title 18 of the US-code and that such willful false statements may jeopardize the validity of the above-identified patent issued thereon.

Ludwigshafen, October 20, 2006

.....
(Wilfried Braje)

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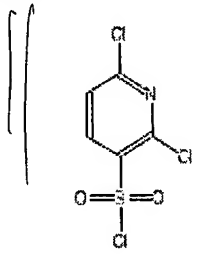

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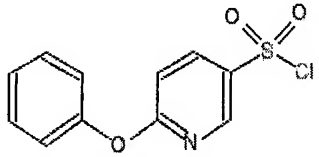

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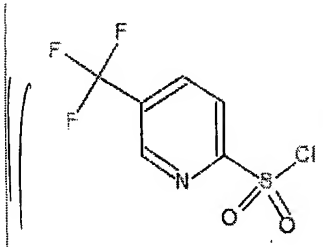

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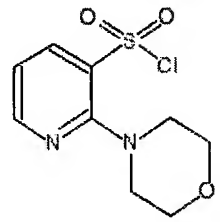

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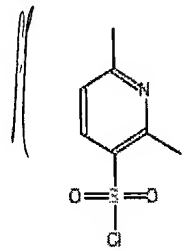

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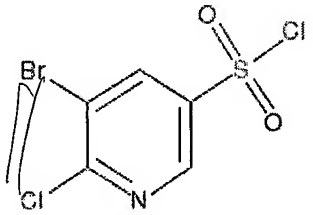

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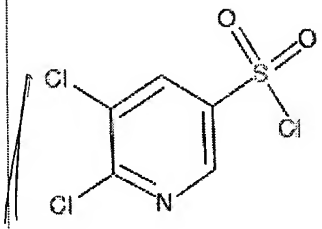

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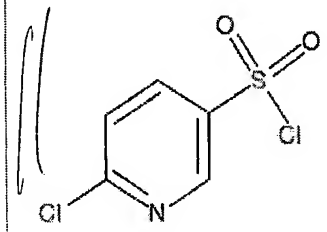

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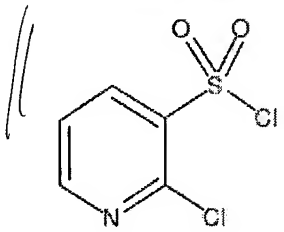

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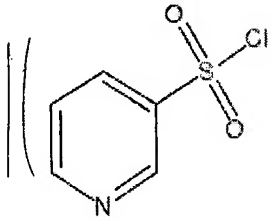

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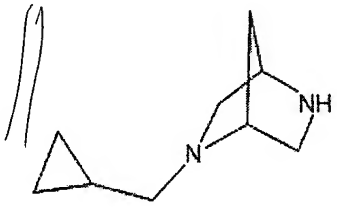

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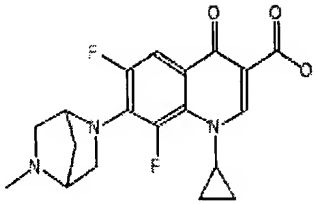

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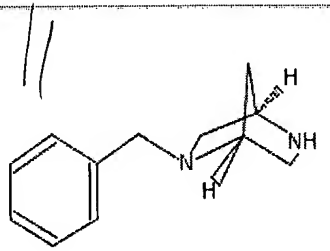

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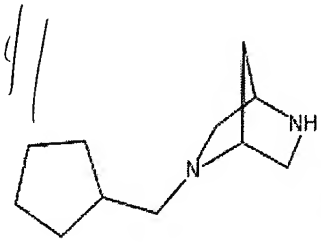

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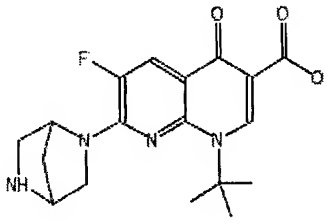

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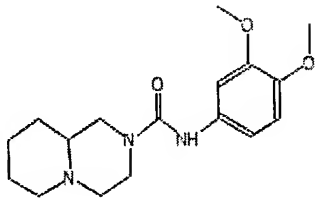

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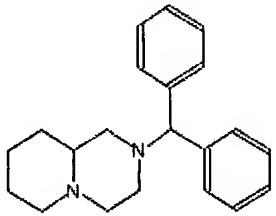

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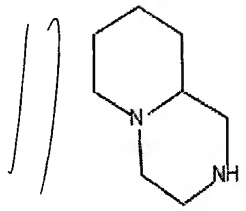

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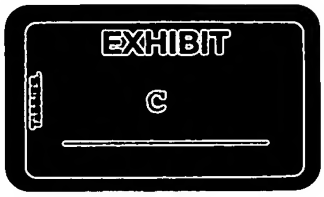
Polish Journal of Pharmacology
Pol. J. Pharmacol., 2003, 55, 449–454
ISSN 1230-6002



PRELIMINARY COMMUNICATION

ANXIOLYTIC-LIKE EFFECTS OF PREFERENTIAL DOPAMINE D_3 RECEPTOR AGONISTS IN AN ANIMAL MODEL

Zofia Rogóż[1,#], Grażyna Skuza[1], Aleksandra Kłodzińska[2]

[1]Department of Pharmacology, [2]Department of New Drug Research, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Anxiolytic-like effects of preferential dopamine D_3 receptor agonists in an animal model. Z. ROGÓŻ, G. SKUZA, A. KŁODZIŃSKA. Pol. J. Pharmacol., 2003, 55, 449–454.

The aim of the present study was to examine a potential anxiolytic-like action of (+)-7-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (7-OH-DPAT), a preferential dopamine D_3 receptor agonist, and (N-[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]-2-naphthylcarboxamide (BP 897), partial dopamine D_3 receptor agonist. Diazepam was used as a reference compound. The anxiolytic-like effect of those drugs was tested in the conflict drinking test (Vogel test) in male Wistar rats. The obtained results showed that 7-OH-DPAT and BP 897 (like diazepam) induced anxiolytic-like effects in the conflict drinking test. 7-OH-DPAT (0.05 and 0.1 mg/kg), BP 897 (0.5 mg/kg) and diazepam (5 and 10 mg/kg), tested at the effective doses in an animal model, did not affect motor coordination but produced significant reduction in exploratory activity in the open field test. These data suggest that preferential dopamine D_3 agonists may play a role in the therapy of anxiety, however, further studies are necessary to elucidate the mechanism of these actions.

Key words: *7-OH-DPAT, BP 897, conflict drinking test, anxiety, rats*

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INTRODUCTION

Behavioral and biochemical data indicate the involvement of dopamine (DA) neurotransmitter system in pathophysiology of anxiety. It is well established that stress activates the mesocorticolimbic DA system and increases extracellular DA level in the nucleus accumbens septi and medial prefrontal cortex, inducing anxiolytic-like behavioral effects [3, 6, 21, 22]. There is also evidence that stress-induced increases in DA metabolism can be attenuated by antianxiety drugs, such as diazepam and ICS 205930 [7, 11]. However, behavioral evidences of the involvement of D_1 receptors in anxiety are week and inconsistent [1, 18]. On the other hand, animal studies indicated the anxiolytic-like effects of D_2 receptor antagonists such as haloperidol or sulpiride [4, 15]. Also D_2 agonists have been discussed to be involved in anxiety because low doses of apomorphine or quinpirole exhibited anxiolytic-like effects in animals whereas their higher doses produced anxiogenic-like effects [8, 22]. Biochemical studies have indicated that haloperidol, sulpiride and quinpirole show affinity not only for D_2 and also for D_3 receptors [23].

The D_3 receptor, which was cloned and its neuroanatomical distribution was identified in 1990, is principally located in the limbic projection areas associated with cognitive, emotional and endocrine functions [2, 23]. It has been demonstrated that mice without functional D_3 receptors show the reduced anxiety in the open field and plus-maze tests [24]. Putative D_3 receptor antagonists such as PNU 99194A and nafadotride have been found to have antianxiety effects in the conflict drinking test in rats and exploration models in rats or mice [9, 19, 20]. Also D_3 receptor agonists, used at low doses, have been suggested to be involved in modulation anxiety level [1].

The present study was carried out to determine whether (+)-7-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (7-OH-DPAT), preferential D_3 receptor agonist, which has been found to possess 10- to 200-fold greater preference for D_3 than for D_2 receptors [5, 10, 13], and (N-[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]-2-naphthylcarboxamide (BP 897), a partial D_3 receptor agonist [16], whose antianxiety effect has not been studied as yet, induce anxiolytic effect in the conflict drinking test in rats.

MATERIALS and METHODS

The experiments were carried out on male Wistar rats (250–270 g). The animals had free access to food and water and were kept at a constant room temperature (21 ± 1°C) under a 12 h ligh/dark cycle (light on at 07.00 h). 7-OH-DPAT (0.01, 0.05, 0.1 mg/kg *sc*) and BP 897 (0.1, 0.25, 0.5 mg/kg *ip*) were dissolved in saline. Both drugs were injected in a volume of 2 ml/kg 30 min before the test. Diazepam (2.5, 5 and 10 mg/kg) used as a reference compound (suspended in a 1% aqueous solution of Tween 80) was given *ip* 60 min before the test. Each experimental group consisted of 6–8 naive animals/dose, and the animals were used only once in each test. The experiments were performed by an observer blind to the treatment. All experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Substances

(+)-7-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (7-OH-DPAT) was purchased form Research Biochemicals Int. (USA), (N-[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]-2-naphthylcarboxamide (BP 897) was synthesized by Dr. J. Boksa, Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland, and diazepam was obtained from Polfa, Poznań (Poland).

Data analysis

The data were evaluated by a one-way analysis of variance (ANOVA), followed, when appropriate, by individual comparisons with the control using Dunnett's test.

Conflict drinking test (Vogel test)

A modification of the method of Vogel et al. [25], described below, was used. On the first day of the experiment, the rats were adapted to the test chamber for 10 min. It was a plexiglass box (27 × 27 × 50 cm), equipped with a grid floor of stainless steel bars and a drinking bottle containing tap water. After the adaptation period, the animals were deprived of water for 24 h, and then they were placed in the test chamber for another 10-min adaptation period, during which they had free access to the drinking bottle. Afterwards, they were allowed a 30-min free-drinking session in their home cage.

After another 24-h water deprivation period, the rats were again placed in the test chamber and allowed to drink for 30 s. Immediately afterwards, drinking attempts were punished with an electric shock (0.5 mA). The impulses were released every 2 s (timed from the moment when a preceding shock was delivered), between the grid floor and the spout of the drinking bottle. Each shock lasted 1 s, and if the rat was drinking when an impulse was released, it received a shock. The number of shocks accepted throughout a 5-min experimental session was recorded.

Shock threshold and free-drinking tests in rats

In order to account for the possibility of drug-induced changes in the perception of a stimulus or in the thirst drive, which might have contributed to the activity in the conflict drinking test, stimulus threshold measurements and a free-drinking experiment were also carried out. In both cases, the rats were treated before the experiment in the same manner as that described in the conflict drinking test, including two 24-h water deprivation periods separated by 30 min of water availability. In the shock threshold test, the rats were placed individually in the box, and electric shocks were delivered through the grid floor. The shock threshold was determined stepwise with 15 s shock-free intervals by increasing manually the current (0.1, 0.2, 0.3, 0.4, 0.5 mA). The shock lasted for 1 s and was delivered through the grid floor until a rat showed an avoidance reaction to an electric stimulus (jump or jerk).

In the free-drinking test, each animal was allowed to drink from the water spout. Licking was not punished. The total amount of water (ml), consumed within 5 min, was recorded for each rat.

Open field test

The studies were carried out on rats according to a slightly modified method of Janssen et al. [12]. The center of the open arena (1 m in diameter), divided into six symmetrical sectors without walls, was illuminated with a 75 W electric bulb hung directly 75 cm above it. During all the experiments, the laboratory room was dark. Individual control or drug-injected animals were placed gently in the center of the arena and were allowed to explore it freely. The time of walking, ambulation (the number of crossings of sector lines) and the number of rearing and peeping episodes (looking under the edge of the arena) were recorded for 5 min.

Rota-rod test

Rats were preselected 1 h before the test on the rotating rod (8 cm in diameter, 6 r.p.m.). Those staying on the rotating road for 2 min (approximately 95% of animals) were placed again on the same rotating rod after drug administration and were observed for 2 min. The number of animals falling from the rota-rod within 2 min was recorded.

RESULTS and DISCUSSION

An anxiolytic-like effect of preferential D_3 receptor agonist, 7-OH-DPAT and BP 897, was evaluated in the conflict drinking test (Vogel test). The Vogel test in rats [25] is a procedure widely employed as a screening method for anxiolytics,

Table 1. Effect of 7-OH-DPAT, BP 897 and diazepam on the conflict drinking test in rats

Compounds (mg/kg)	Number of shock accepted /5 min (mean ± SEM)
Vehicle	9.1 ± 1.0
7-OH-DPAT 0.01	11.0 ± 2.2
7-OH-DPAT 0.05	43.5 ± 3.9*
7-OH-DPAT 0.1	32.0 ± 4.6*
	F(3, 28) = 26.33
	α = 0.001
Vehicle	7.0 ± 1.2
BP 897 0.1	13.0 ± 4.5
BP 897 0.25	20.9 ± 2.3
BP 897 0.5	41.5 ± 6.2*
	F(3, 27) = 13.65
	α = 0.001
Vehicle	9.3 ± 2.1
Diazepam 2.5	23.1 ± 3.5
Diazepam 5	37.6 ± 6.1*
Diazepam 10	52.8 ± 5.7*
	F(3, 24) = 14.79
	α = 0.001

7-OH-DPAT (*sc*), BP 897 (*ip*) were administered 30 min, diazepam (*ip*) 60 min before the test. The values represent mean ± SEM (n = 6–8 rats per group) of the number of shocks accepted during a 5 min experimental session; * $p < 0.001$ (ANOVA followed by Dunnett's test) *vs.* vehicle-treated group

and it is considered to be one of the most specific screening methods for these drugs.

Our results indicate that 7-OH-DPAT at doses of 0.05 and 0.1 mg/kg (but not at 0.01 mg/kg) significantly increased the number of shocks accepted during the experimental session in the Vogel test (Tab. 1). BP 897 showed an antianxiety-like effect only at the higher dose of 0.5 mg/kg, significantly increasing the number of shocks accepted but its lower doses (0.1 or 0.25 mg/kg) were inactive in this test (Tab. 1). Diazepam (2.5–10 mg/kg), used as a reference drug, significantly and dose-dependently increased the number of accepted shocks, the maximum effect having been observed after the administration of 10 mg/kg of the drug (Tab. 1). The effect of 7-OH-DPAT (0.05 mg/kg) or BP (0.5 mg/kg) was comparable to that evoked by diazepam at a dose of 5 mg/kg (Tab. 1). The possibility that the efficacy of effective dose of 7-OH-DPAT or BP 897 was dependent upon the reduced perception of the stimulus or to an increased thirst drive was excluded since 7-OH-DPAT or BP 897, tested at the effective doses in the conflict drinking test, increased neither the threshold current nor water intake (in the case of BP 897) but 7-OH-DPAT even decreased it (Tab. 2). 7-OH-DPAT (0.05 or 0.1 mg/kg) and BP 897 (0.5 mg/kg) at doses effective in the conflict drinking test did not disturb motor coordination tested in the rota-rod test in rats (data not shown).

Administration of 7-OH-DPAT at doses of 0.05 or 0.1 mg/kg and BP 897 at the dose of 0.5 mg/kg (but not at 0.25 mg/kg) produced significant reduction in exploratory activity of rats, as evaluated in the open field test (Tab. 3). Diazepam, used as a reference drug, at doses of 2.5, 5 and 10 mg/kg evoked a dose-dependent and statistically signifi-

Table 2. Effects of 7-OH-DPAT and BP 897 on the shock threshold and amount of water consumed by water deprived rats

Compounds (mg/kg)	Shock threshold (mA) (mean ± SEM)	Water consumption (ml) (mean ± SEM)
Vehicle	0.4 ± 0.02	10.7 ± 0.5
7-OH-DPAT 0.05	0.4 ± 0.02	7.5 ± 0.7*
7-OH-DPAT 0.1	0.4 ± 0.03	6.9 ± 0.5*
BP 897 0.25	0.4 ± 0.04	10.5 ± 0.4
BP 897 0.5	0.4 ± 0.03	10.1 ± 0.5
	F(4, 25) = 0.27	F(4, 25) = 12.27
	ns	$\alpha = 0.001$

7-OH-DPAT (*sc*), BP 897 (*ip*) were given 30 min before the test. The animals were observed for 5 min. The values represent mean ± SEM (n = 6 rats per group), * $p < 0.001$ (ANOVA followed by Dunnett's test) *vs.* vehicle-treated group

Table 3. Effect of 7-OH-DPAT, BP 897 and diazepam on exploratory activity in the open field test in rats

Compounds (mg/kg)	Exploratory activity (mean ± SEM)		
	Time of walking	Ambulation	Peeping + rearing
Vehicle	42.0 ± 2.1	18.0 ± 1.4	16.6 ± 1.1
7-OH-DPAT 0.05	14.0 ± 1.5*	7.1 ± 0.7*	6.1 ± 0.8*
7-OH-DPAT 0.1	19.6 ± 1.6*	8.0 ± 0.7*	8.1 ± 0.7*
BP 897 0.25	37.3 ± 1.9	13.6 ± 1.0	12.3 ± 1.4
BP 897 0.5	33.4 ± 1.3*	11.5 ± 1.7*	13.1 ± 0.7
	F(4, 35) = 48.60	F(4, 35) = 14.66	F(4, 35) = 18.00
	$\alpha = 0.001$	$\alpha = 0.001$	$\alpha = 0.001$
Vehicle	37.7 ± 3.8	14.2 ± 1.9	11.2 ± 1.2
Diazepam 2.5	28.3 ± 3.8	11.3 ± 1.2	9.2 ± 1.5
Diazepam 5	17.0 ± 1.9*	5.3 ± 0.5*	4.8 ± 0.6*
Diazepam 10	9.8 ± 0.9*	4.3 ± 0.6*	3.8 ± 0.6*
	F(3, 20) = 18.16	F(3, 20) = 15.55	F(3, 20) = 8.33
	$\alpha = 0.001$	$\alpha = 0.001$	$\alpha = 0.001$

7-OH-DPAT (*sc*), BP 897 (*ip*) were given 30 min and diazepam (*ip*) 60 min before the test. The animals were observed for 5 min. The values represent mean ± SEM (n = 6–8 rats per group) * $p < 0.001$ (ANOVA followed by Dunnett's test) *vs.* vehicle treated group

cant reduction in exploratory activity (time of walking, ambulation and peeping + rearing episodes, Tab. 3) in rats. The results showed that an anxiolytic-like effect induced by 7-OH-DPAT, BP 897 or diazepam, evaluated in the conflict drinking test, as an increase in a number of accepted shocks, was not connected with reduction in exploratory activity in the open field test in rats.

An involvement of a preferential D_3 receptor agonist in psychiatric disorders, such as anxiety, has been suggested by other authors [1, 17]. Anxiolytic-like effects of (±)-7-OH-DPAT were observed in the elevated plus-maze paradigm in mice [17] and in the vocalization test in rats [1]. In the latter test, only D_3 receptor agonists at low doses inhibited ultrasonic vocalization with the following ED_{50} values: quinpirole (0.04 mg/kg), pramipexole (0.09 mg/kg), roxindole (0.04 mg/kg), talipexole (0.04 mg/kg), (±)-7-OH-DPAT (0.05 mg/kg) and PD 128907 (0.13 mg/kg) [1]. The D_2 (haloperidol, raclopride) and D_3 (U99194A, S(–)DS121) receptor antagonists and D_1 agonist (R(+)-SKF 38393) or antagonist (SCH 23390) and DA uptake inhibitors (GBR 12909, GBR 12935) laced significant inhibitory effects on ultrasonic vocalization [1].

The above data indicated that preferential D_3 receptor agonists, such as 7-OH-DPAT or BP 897, at low doses, produced an anxiolytic-like effect, and suggested the involvement of D_3 receptors in pathomechanism of anxiety. Future studies are necessary to elucidate the mechanism of these effects using the selective D_3 antagonists, such as S33084 [14].

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Received: March 26, 2003; in revised form: April 25, 2003.

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Brain Research Reviews 49 (2005) 77–105
BRAIN RESEARCH REVIEWS
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Review

The role of central dopamine D_3 receptors in drug addiction: a review of pharmacological evidence

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Accepted 6 December 2004
Available online 11 February 2005

Abstract

The cDNA for the dopamine D_3 receptor was isolated and characterized in 1990. Subsequent studies have indicated that D_3 receptors, as well as D_3 receptor mRNA, are primarily localized in limbic regions in mammals. This finding led to the postulate that D_3 receptors may be involved in drug dependence and addiction. However, this hypothesis has been difficult to test due to the lack of compounds with high selectivity for central D_3 receptors. The interpretation of results from studies using mixed D_2/D_3 agonists and/or antagonists is problematic because these agents have low selectivity for D_3 over D_2 receptors and it is likely that their actions are primarily related to D_2 receptor antagonism and possibly interaction with other neurotransmitter receptors. Currently, with the synthesis and characterization of new highly selective D_3 receptor antagonists such as SB-277011-A this difficulty has been surmounted. The purpose of the present article is to review, for the first time, the effects of various putative D_3 receptor selective compounds in animal models of drug dependence and addiction. The results obtained with highly selective D_3 receptor antagonists such as SB-277011-A, SB-414796, and NGB-2904 indicate that central D_3 receptors may play an important role in drug-induced reward, drug-taking, and cue-, drug-, and stress-induced reinstatement of drug-seeking behavior. Provided these results can be extrapolated to human drug addicts, they suggest that selective DA D_3 receptor antagonists may prove effective as potential pharmacotherapeutic agents to manage drug dependence and addiction.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Behavioral pharmacology

Keywords: Addiction; Brain stimulation reward; Conditioned place preference; Dopamine D_3 receptors; SB-277011-A; Self-administration

Contents

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0165-0173/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainresrev.2004.12.033

1. Introduction: drug addiction, the mesolimbic DA system, and the DA D_3 receptor

Drug addiction is a dynamic phenomenon characterized by several key stages: (1) initiation or acquisition of drug-taking, (2) compulsive drug taking, and (3) drug taking coupled with a marked narrowing of the behavioral repertoire. The behavioral progression typically ends with excessive drug intake, loss of control over intake, and vulnerability to relapse [156]. One of the main challenges in drug dependence research is to understand the psychobiological dysregulation, and by extension, the molecular, cellular, and system processes that underlie these various phases.

In order to assess the neuroadaptations occurring within the brain reward systems in response to acute and repeated exposure to drugs of abuse, one must first understand the neurobiological bases of drug reward [183]. Consequently, molecular and cellular approaches have emphasized differences in the primary sites of action of drugs of abuse [52]. In contrast, the neural systems approach has explored the possible commonalities of the effects of such drugs [213]. The major focus of most of these investigations has been the mesocorticolimbic dopamine (DA) system originating from the ventral tegmental area (VTA) and projecting towards a wide range of limbic and telencephalic structures including the olfactory tubercle, the amygdala, frontal and limbic cortices, especially the medial prefrontal cortex (mPFC), and the nucleus accumbens (NAc). The NAc occupies a prominent position in the ventral striatum and is a main target of the mesotelencephalic DA system. As such, DA neurotransmission in the NAc provides the framework for theories exploring the chemoarchitectural substrates of reward and motivation, including aspects of drug addiction.

The role of mesolimbic DA in general reinforcement processes clearly pointed towards DA receptors as potential targets for the study of drug consumption and craving. After the cloning of the D_1 and D_2 receptors [39,204,315] several additional low-abundance DA receptors were identified. These new subtypes included the D_3 and D_4 receptors, which are homologous to the D_2 receptor, and the D_5 receptor, which is homologous to the D_1 receptor [273,286,292]. A growing body of evidence suggests strongly that the DA D_3 receptor is significantly involved in mechanisms of drug dependence and abuse [6,10,41,43,84,131,167,295]. These findings have underlined the need for selective tools to investigate the role of the DA D_3 receptor in drug dependence. The present review will not cover molecular biological (gene organization, receptor synthesis, receptor isoforms, and protein structure) or cellular signaling mechanisms associated with the DA D_3 or other DA receptor subtypes (see [175]), but will specifically focus on the rationale for the use of selective DA D_3 receptor antagonists as potential pharmacotherapeutic agents to manage drug dependence and addiction. The review will start with a discussion of the selective distribution of the D_3 receptor in the mesolimbic DA system. Functional pharmacological aspects of different DA D_3 and mixed D_3/D_2 receptor agonists and antagonists will then be summarized in the context of drug addiction paradigms. Finally, potential sites of action of DA D_3 receptor antagonists in the brain will be discussed.

2. Localization of DA D_3 receptors

The DA D_3 receptor was initially cloned from a rat cDNA library by using probes derived from the DA D_2 receptor sequence [273]. The cloning of the human D_3 receptor was reported thereafter [112], followed by the murine D_3 receptor [100]. Molecular neurobiology techniques permitted the study of the D_3 receptor in vitro by transfection in cells that do not normally express DA receptors. Molecular methods also allowed the study of receptor messenger ribonucleic acid (mRNA) in the brain.

2.1. Rodent DA D_3 receptor localization under basal condition

The greatest densities of D_3 mRNA in rat brain are found primarily in limbic brain areas such as the NAc (rostral pole and parts of shell), islands of Calleja, and olfactory tubercle [30,66,77,78,162,197,233,273]. Other brain areas reported to contain high levels of D_3 mRNA include the medial and ventral lateral geniculate nuclei, mammillary nucleus, magnocellular preoptic nucleus, lateral substantia nigra pars compacta, dorsal cochlear nuclei, Purkinje cell layer of the vestibulocerebellum, paracentral thalamic nucleus, bed nucleus of the stria terminalis (BNST), and vertical limb of the diagonal band of Broca [30,77,197]. Bouthenet et al. [30] have reported moderate levels of D_3 mRNA in the amygdala, ventral pallidum, various thalamic and hypothalamic nuclei, superior colliculus, inferior olivary nucleus, and nucleus of the horizontal limb of the diagonal band of Broca. Transcripts for the D_3 receptor are located in the mesencephalic areas rich in DA cell bodies [77,78,282]. The restricted localization of the D_3 receptor has led to the hypothesis that it may play an important role in emotion, cognition, and addiction [117,167,231,232,253,273,275].

In vitro homogenate and autoradiographic studies indicate that DA D_3 receptors are expressed in highest densities in the islands of Calleja, olfactory bulb, NAc, and intermediate lobe of the pituitary [20,27,106,107,117,162, 173,177,179,182,252,258,282] (for reviews, see Refs. [175,258]). The lateral aspect of the substantia nigra pars compacta and the molecular layer of the vestibulocerebellum also contain moderate levels of D_3 receptors.

2.2. Rodent DA D_3 receptor localization following drug exposure

DA D_3 mRNA and receptors are increased in cocaine cue conditioned locomotion [168]. A recent series of experiments has also shown that termination of a cocaine self-administration regimen increases DA D_3 binding over time in the NAc core and ventral caudate–putamen, an adaptive change that may occur through regulatory responses to an increase in phasic DA levels associated with cocaine-taking and -seeking behavior [212]. In addition, nicotine-induced conditioned locomotion [169] and nicotine behavioral sensitization [170] are both associated with significant increases in D_3 receptor binding and mRNA levels in the NAc shell without altering D_1 or D_2 receptor mRNA in the NAc shell or core subregions. Furthermore, twice daily morphine administration over 8 consecutive days with an escalating dosing regimen starting at 10 mg/kg was shown to produce a significant increase in D_3 receptor mRNA in the caudate–putamen and ventral midbrain, including the substantia nigra and VTA. Morphine also produced a 25% decrease in D_2 receptor mRNA in the caudate–putamen, but no alterations were seen in mRNA levels related to tyrosine hydroxylase or the DA transporter [278].

2.3. Human DA D_3 receptor localization under basal condition

Expression of DA D_3 receptor mRNA in the human brain follows a similar pattern as in the rodent brain [120,162, 194,287]. High levels are present in the islands of Calleja, ventral striatum/NAc, dentate gyrus, and striate cortex. Low to moderate densities are present in the caudate–putamen, anterior and medial thalamic nuclei, amygdala, hippocampal CA region, cortical regions (particularly the anterior cingulate and subcallosal gyrus), lateral geniculate body, substantia nigra pars compacta, locus ceruleus, and median raphé. Receptor binding data also indicate the presence of D_3 receptors in the NAc, internal globus pallidus, ventral pallidum, septum, islands of Calleja, amygdala, and VTA [120,124,132,162,205]. Interestingly, significant inter-species differences have been reported in the distribution of D_3 receptors [176]. For example, although rats, mice, guinea pigs, and humans show similar distributions of D_3 sites in the basal ganglia and limbic forebrain, notable differences are typically observed in hypothalamic, thalamic, and mesencephalic brain areas. Furthermore, the detection of D_3 sites in the vestibulocerebellum of the rat, but not other species suggests a possible role of DA D_3 receptors in the control of posture, muscle tone, or eye movements in the rat.

2.4. Human DA D_3 receptor localization following drug exposure

Postmortem studies using [^{3}H]-(+)-7-OH-DPAT have shown an increase in the number of D_3 receptors in the NAc [190,256] and specific areas of the striatum and substantia nigra [281] in cocaine overdose fatalities compared to drug-free and age-matched controls. A significant elevation in the levels of D_3 receptor mRNA of human cocaine fatalities has also been reported [256] but was not corroborated by another study [195]. The discrepancy between these two studies might be related to the different methods used to determine levels of D_3 receptor mRNA (reverse transcription-polymerase chain reaction (RT-PCR) [256] vs. in situ hybridization [195]). A human postmortem study comparing DA receptor density between smokers and nonsmokers revealed no significant differences in D_3 receptors [63]. In

that study, analysis of groups and areas for D_3 receptor binding in the striatum indicated a significant difference between areas, but no difference between smokers, ex-smokers and nonsmokers was observed and there was no interaction between groups and areas. However, [^{3}H]-7-OH-DPAT binding was focused mainly on the putamen and caudate rather than the NAc. A recent study measured the expression of dopamine D_3 receptor mRNA in peripheral blood lymphocytes by real-time polymerase chain reaction in smokers, former smokers, and nonsmoking control subjects [69]. The results of this study revealed a significant reduction in DA D_3 receptor mRNA expression in smokers, but not former smokers compared with controls. Furthermore, the expression of DA D_3 receptor mRNA in smokers was negatively correlated with daily number of cigarettes, suggesting a selective inhibiting effect of smoking on the expression of DA D_3 receptor mRNA. Additional studies looking at the density of DA D_3 receptors in smokers vs. nonsmokers are clearly warranted.

The above findings suggest that the distribution pattern of DA D_3 receptors in both rodent and human brain is compatible with a major role in emotion, cognition, and processing of motor and sensory information. Postmortem and preclinical studies point to the possibility that chronic abuse of cocaine, nicotine, and opioids may be associated with an adaptive change in D_3 receptors.

3. In vitro pharmacological characterization of DA D_3 ligands

A number of agonists and antagonists have been used to characterize the pharmacology of DA D_3 receptors in various expression systems and in the brain. However, results generated from these studies vary considerably and seem to be dependent upon the expression system or tissue, the radioligand, and the in vitro assay conditions used (for a detailed review, see Ref. [175]). For example, the observed D_3 selectivity of several DA agonists may directly result from the use of in vitro conditions that disfavor the high-affinity conformation of the D_2 receptor such as the inclusion of Na^+ in some in vitro assay systems [40,180]. The greatest D_2/D_3 selectivity with [^{3}H]-7-OH-DPAT and [^{3}H]-PD128907 can also be obtained in the absence of Mg^{2+} and the presence of EDTA [40]. Studies have shown that although selective labeling of putative D_3 sites may be reasonably obtained using [^{3}H]-PD128907 or [^{3}H]-7-OH-DPAT, labeling of the D_2 site is also observed [20,114,250] as well as labeling of σ [250,296] and 5-HT_{1A} receptors [72,198] in rat brain. In our hands, however, [^{3}H]-7-OH-DPAT binding to rat brain slice preparations can be fully displaced by the highly selective DA D_3 receptor antagonist SB-277011-A, at doses predicted by the affinity values of [^{3}H]-7-OH-DPAT and SB-277011-A for rat D_2 and D_3 receptors [182,229], suggesting that appropriate conditions can be chosen to favor preferential [^{3}H]-7-OH-DPAT binding to D_3 receptors in receptor autoradiography experiments (see Fig. 1 for further details).

In addition to radioligand binding studies, several functional assays including induction of Chinese hamster ovary (CHO) cell mitogenesis, melanocyte aggregation, or extracellular acidification rates in the microphysiometer assay have not only established the agonist or antagonist activity of a variety of DA compounds at the D_3 receptor, but have also contributed to determining their D_2/D_3 selectivity. In contrast with the significant DA D_3 selectivity reported in some binding studies, agonists such as DA, PD128907, 7-OH-

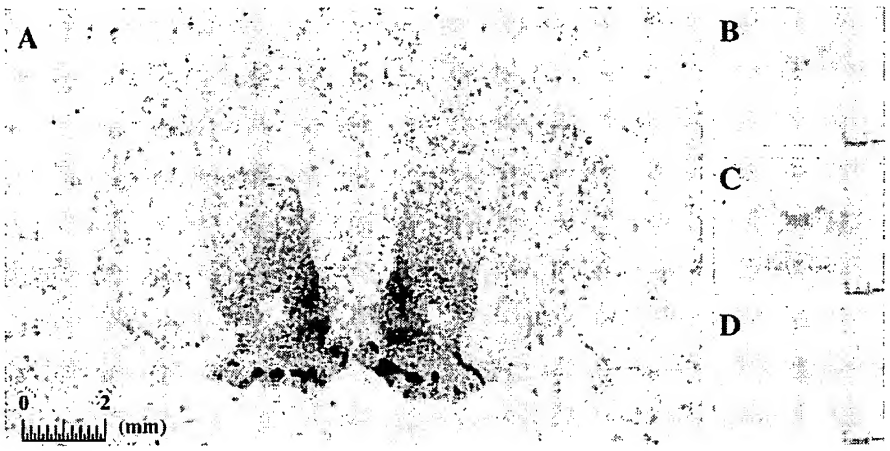


Fig. 1. Distribution of D_3 receptors in rat brain, as revealed by [^{3}H]-7-OH-DPAT autoradiography—displacement with the selective DA D_3 receptor antagonist SB-277011. Coronal rat brain sections (at +1.70 mm from Bregma) were incubated in the presence of 0.5 nM [^{3}H]7-OH-DPAT for 60 min at room temperature. (A) Total binding; (B) nonspecific binding, in the presence of 1 μM dopamine; (C) binding in the presence of 100 nM SB-277011-A; (D) binding in the presence of 1 μM SB-277011-A. Calibration bars correspond to 2 mm. Incubation with [^{3}H]-7-OH-DPAT was performed in the presence of 1 mM EDTA, in order to abolish the radioligand binding to D_2 receptors without affecting that to D_3 receptors, as demonstrated in transfected cell lines [182]. The specificity of the radioligand binding to D_3 receptors was confirmed by the distribution of [^{3}H]-7-OH-DPAT binding sites, in line with that of D_3 mRNA [30] and by displacement with the selective D_3 antagonist SB-277011-A. As predicted by the affinity values of [^{3}H]7-OH-DPAT and SB-277011-A for rat D_2 and D_3 receptors [182,229], 0.5 nM [^{3}H]7-OH-DPAT binding was inhibited by 80% and 100% by 100 nM and 1 μM SB-277011-A, respectively.

DPAT, quinelorane, and (+)-UH232 exhibited only modest, if any, D_3 selectivity in these functional tests [56,225,226] (for a direct comparison of selectivities obtained from radioligand binding vs. functional assays in CHO cells using the cytosensor microphysiometer, the reader is referred to Ref. [60]).

Overall, these findings clearly demonstrate that the rank order of potencies and selectivities in functional assays is not equivalent to the rank order of radioligand binding affinities and selectivities. These results further emphasize the need to consider both radioligand binding affinities and functional potencies as well as intrinsic activities in order to reliably interpret behavioral effects mediated by DA D_2 and D_3 receptors. Furthermore, they also further underline the need for caution in the use of in vitro binding data in the interpretation of in vivo or in vitro functional studies.

4. Role of DA D_3 receptors in drug addiction: studies with mixed DA D_2/D_3 receptor agonists

The mixed D_2/D_3 agonists 7-OH-DPAT [41,42,102,219], quinpirole [41], quinelorane [43,219], pramipexole [43], and PD128907 [43] have all been shown to decrease cocaine self-administration in rats. However, the same mixed D_2/D_3 agonists have also led to discrepant findings in a wide range of paradigms. For example, 7-OH-DPAT (0.1 mg/kg) inhibits cocaine-seeking behavior as assessed by the conditioned place preference (CPP) paradigm [150] but reinstates intravenous (iv) cocaine self-administration behavior at doses of 3 and 10 mg/kg [257]. In contrast, Khroyan et al. [151] found that neither 7-OH-DPAT (0.01–0.1 mg/kg) nor PD128907 (0.01–0.1 mg/kg) alter cocaine-triggered reinstatement of drug-seeking behavior. Furthermore, 7-OH-DPAT (2.5–74 μg/kg) does not significantly alter brain stimulation reward (BSR) maintained by electrodes in the lateral hypothalamus in rats [128], and 7-OH-DPAT (0.05 mg/kg) given subcutaneously (sc) does not block the development of sensitization to cocaine [191]. However, 7-OH-DPAT can partially substitute for cocaine in the drug discrimination paradigm [1,105,146,161,208,269,280].

These mixed in vivo findings clearly demonstrate that the effect of the aforementioned mixed D_2/D_3 agonists (see Table 1) in drug addiction paradigms might be related to (1) their lack of selectivity for D_3 over D_2 receptors and/or (2) their ability to have incentive value per se. In support of the first argument (lack of selectivity) are the findings described in the previous section (i.e., radioligand binding studies vs. in vitro functional assays). In addition to lack of D_3 receptor selectivity under in vitro assay conditions, both 7-OH-DPAT and PD128907 may activate D_2 receptors in vivo in rats as a function of the doses used in different behavioral paradigms [2,19,36,71,73,95,146,159,180,181,203,228,293]. For example, behavioral characterizations of both 7-OH-DPAT and PD128907 up to 10 mg/kg revealed U-shaped dose–response curves for both compounds [2,71,228], suggesting activation of D_3 receptors at low doses and increasing D_2 receptor occupancy at higher doses. This hypothesis is further supported by studies based upon D_2 receptor protection from *N*-ethoxycarbonyl-2-ethoxyl-1,2-dihydroquinilone (EEDQ) alkylation, suggesting that 7-OH-DPAT doses below 0.3 mg/kg are devoid of significant D_2 receptor occupancy [181]. Similarly, studies using DA D_3 knockout mice showed that intraperitoneal (ip) doses of PD128907 in the range of 0.03–0.1 mg/kg affect DA release in wild type, but not knockout mice, suggesting D_3-mediated effects of PD128907 when given at sufficiently low dose [314]. Furthermore, 7-OH-DPAT and PD128907 doses below 10 μg/kg have been reported to produce inhibition of novelty-stimulated locomotion in wild type, but not in D_3 receptor knockout mice [227]. All other D_3 knockout studies using 7-OH-DPAT or PD128907 doses at least 10-fold higher reported that neither 7-OH-DPAT nor PD128907 inhibit locomotion through selective D_3 receptor stimulation [28,29], and hence concluded that locomotor inhibitory affects of 7-OH-DPAT are mediated through D_2 autoreceptors or other receptors.

In support of the second argument (incentive value) is that 7-OH-DPAT alone (at 5 mg/kg [187]) or PD128907 alone (at 1 mg/kg [148]) have been reported to produce a significant CPP (however, see Refs. [147,149,150] for contrary findings). Furthermore, 7-OH-DPAT at 0.03 mg/kg [147] or at 0.05–0.1 mg/kg [121] has been reported to produce conditioned place aversion (CPA), as does PD128907 at 1 mg/kg sc [121]. It has also been shown that 7-OH-DPAT can attenuate the CPP response to morphine [245], D-amphetamine [149], and cocaine [150] in rats. However, evidence from Ukai et al. [290] indicates that administration of *R*(+)-7-OH-DPAT (0.001–0.1 mg/kg sc) produces an amnesic effect in the passive avoidance learning model in mice, and Smith et al. [270] have demonstrated that systemic administration of 7-OH-DPAT (6 or 25 μg/kg) induces cognitive impairment in the marmoset. Consequently, one cannot rule out the possibility that 7-OH-DPAT may interfere with the animals' association of the appetitive value of these agents with the appropriate cues.

Together, these findings clearly indicate that the degree of functional selectivity of mixed D_2/D_3 agonists in recombinant systems is too low to allow meaningful discrimination in vivo between D_2 and D_3 receptors. The significant controversy around the outcome of in vivo studies using mixed DA D_2/D_3 agonists relates essentially to their lack of selectivity, but also to the possibility that these agents have intrinsic rewarding properties or produce nonspecific aversive effects.

5. Role of DA D_3 receptors in drug addiction: studies with the partial DA D_3 receptor agonist BP-897

A series of in vivo studies assessed the efficacy of the partial DA D_3 receptor agonist BP-897 in animal models of drug addiction. In 1999, Pilla and colleagues [224]

Table 1
Representative DA D_2/D_3 receptor agonists and antagonists, the partial agonist BP-897, and selective DA D_3 receptor antagonists[a]

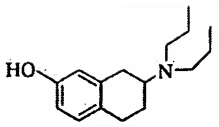
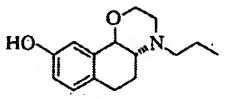
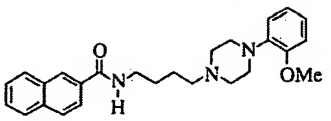
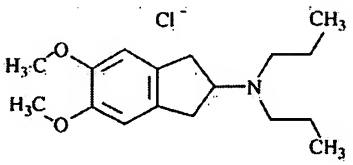
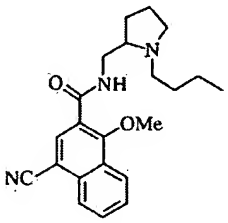
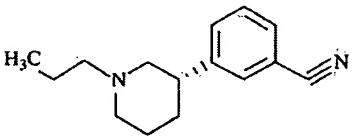
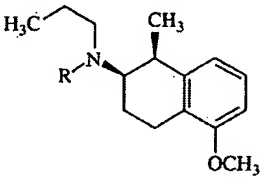
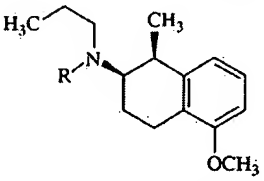
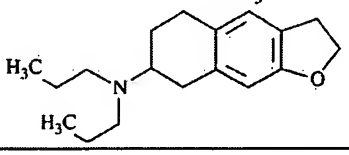
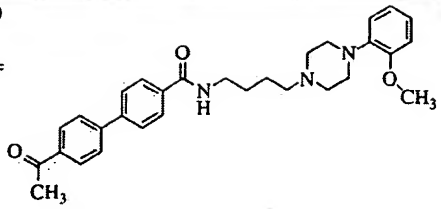
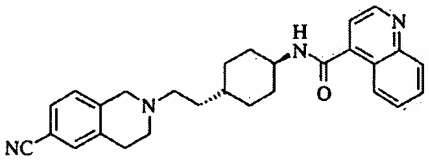
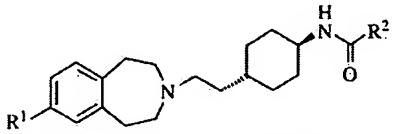
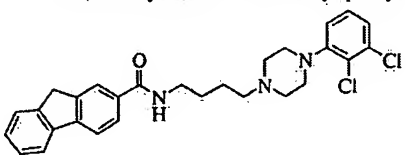
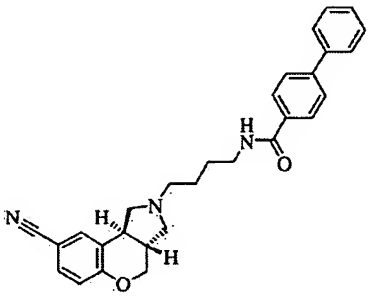
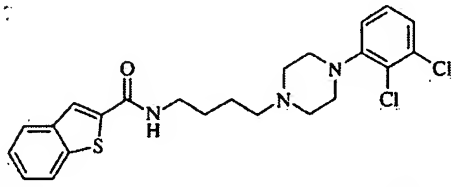
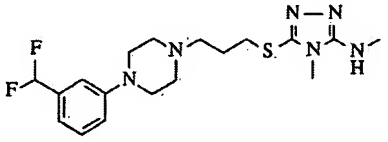
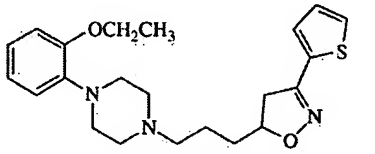
Ligand Name	K_i hD_2 (nM)	K_i hD_3 (nM)	D_2:D_3 *Ki* ratios	Chemical structure
		Mixed DA D_2/D_3 agonists		
7-OH-DPAT[b]	[^{125}I]-iodosulpiride = 92	[^{3}H]-PD128907 = 0.34 [^{3}H]-S14297 = 1 [^{125}I]-iodosulpiride = 2.2	[^{3}H]-PD128907 = 302 [^{3}H]-S14297 = 103 [^{125}I]-iodosulpiride = 47	
PD 128907[c]	[^{125}I]-iodosulpiride = 339	[^{3}H]-PD128907 = 1.33 [^{3}H]-S14297 = 1.31 [^{125}I]-iodosulpiride = 1.9	[^{3}H]-PD128907 = 340 [^{3}H]-S14297 = 345 [^{125}I]-iodosulpiride = 239	
		Partial DA D_3 agonist		
BP-897[d]	[^{125}I]-iodosulpiride = 61	[^{125}I]-iodosulpiride = 0.92	[^{125}I]-iodosulpiride = 66	
		Mixed DA D_2/D_3 antagonists		
U 99194-A[e]	[^{125}I]-iodosulpiride = 2281	[^{3}H]-PD128907 = 160 [^{3}H]-S14297 = 180 [^{125}I]-iodosulpiride = 223	[^{3}H]-PD128907 = 14 [^{3}H]-S14297 = 13 [^{125}I]-iodosulpiride = 10	
Nafadotride[f]	[^{125}I]-iodosulpiride = 5	[^{3}H]-PD128907 = 0.52 [^{3}H]-S14297 = 0.88 [^{125}I]-iodosulpiride = 0.81	[^{3}H]-PD128907 = 9 [^{3}H]-S14297 = 6 [^{125}I]-iodosulpiride = 6	
DS 121[g]	[^{3}H]-spiperone = 1140	[^{3}H]-spiperone = 249	D_2:D_3 *Ki* ratios = 4	
(+)-AJ 76[h] *R=H*	[^{125}I]-iodosulpiride = 155	[^{3}H]-PD128907 = 26 [^{3}H]-S14297 = 34 [^{125}I]-iodosulpiride = 70	[^{3}H]-PD128907 = 6 [^{3}H]-S14297 = 5 [^{125}I]-iodosulpiride = 2	
(+)-UH-232[i] *R=propyl*	[^{125}I]-iodosulpiride = 28	[^{3}H]-PD128907 = 3.3 [^{3}H]-S14297 = 5.4 [^{125}I]-iodosulpiride = 7	[^{3}H]-PD128907 = 8 [^{3}H]-S14297 = 5 [^{125}I]-iodosulpiride = 4	
S 14297[j]	[^{125}I]-iodosulpiride = 297	[^{3}H]-PD128907 = 4.9 [^{3}H]-S14297 = 7.4 [^{125}I]-iodosulpiride = 13	[^{3}H]-PD128907 = 61 [^{3}H]-S14297 = 40 [^{125}I]-iodosulpiride = 23	

Table 1 (*continued*)

Ligand Name	K_i hD_2 (nM)	K_i hD_3 (nM)	D_2:D_3 *Ki* ratios	Chemical structure
GR 103691 [k]	[^{125}I]-iodosulpiride = 24	[^{3}H]-PD128907 = 0.4 [^{3}H]-S14297 = 0.4 [^{125}I]-iodosulpiride = 0.4	[^{3}H]-PD128907 = 60 [^{3}H]-S14297 = 60 [^{125}I]-iodosulpiride =	
		Selective DA D_3 antagonists		
SB-277011-A [l]	[^{125}I]-iodosulpiride pK_i = 5.98	[^{125}I]-iodosulpiride pK_i = 7.95	D_2:D_3 *Ki* ratios = 120	
SB-414796 [m]	[^{125}I]-iodosulpiride pK_i = 6.4	[^{125}I]-iodosulpiride pK_i = 8.4	D_2:D_3 *Ki* ratios = 100	 R^1 = $MeSO_2$; R^2 = 3-(5-methyl-1,2,4-oxadiazol-3-yl)phenyl
NGB-2904 [n]	[^{125}I]-IABN = 911	[^{125}I]-IABN = 1.1	D_2:D_3 *Ki* ratios = 830	
S33084 [o]	[^{125}I]iodosulpride; pKi = 7.54 [^{3}H]spiperone; pKi = 7.28 [^{35}S]GTPγS; pKb = 7.75	[^{125}I]iodosulpride; pKi = 9.59 [^{3}H]spiperone; pKi = 9.40 [^{35}S]GTPγS; pKb = 9.61	[^{125}I]iodosulpride = 120 [^{3}H]spiperone = 125 [^{35}S]GTPγS = 66	
FAUC 365 [p]	[^{3}H]spiperone = 3600	[^{3}H]spiperone = 0.5	D_{2L}:D_3 *Ki* ratios = 7	
A-437203 [q]	Ki D_{2L} = 348	Ki = 29	D_{2L}:D_3 *Ki* ratios = 120	
KCH 1110 [r]	[^{3}H]-spiperone = 118.8	[^{3}H]-spiperone = 1.28	D_2:D_3 *Ki* ratios = 90	

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demonstrated that administration of BP-897 (0.05, 0.5, and 1 mg/kg) produces a significant dose-dependent decrease in the number of responses for cocaine in the first, but not the second interval of a second-order schedule of reinforcement without having intrinsic rewarding effects. In support of those findings [224], recent evidence suggests that BP-897 (1 mg/kg) can reduce cocaine-seeking behavior induced by the presentation of stimuli associated with and predictive of cocaine availability after a period of extinction and in the absence of cocaine itself [50]. However, it must be noted that, in contrast with the findings of Pilla et al. [224], a recent report found that, similar to ip administration of the D_1 receptor antagonist SCH-23390 (0.1 and 0.2 mg/kg) or oral (po) administration of the D_2 receptor antagonist haloperidol (0.2 and 0.5 mg/kg), BP-897 (1 mg/kg ip) increased cocaine self-administration under a continuous reinforcement schedule [102].

BP-897 (0.3, 1, or 3 mg/kg ip) reduces cocaine cue-induced hyperlocomotion in Swiss–Webster mice [168] as well as nicotine-induced conditioned locomotion [169] and nicotine-induced behavioral sensitization in rats [170]. Aujla et al. [12] also showed that BP-897 (1 mg/kg) blocked the expression of conditioned locomotor activity to amphetamine (2 mg/kg) in male Wistar rats without altering the acquisition of conditioning or the locomotor activating effect of amphetamine.

BP-897 significantly decreases the discriminative stimulus effects of D-amphetamine and cocaine [21] and attenuates the expression and acquisition of the CPP response to cocaine without altering the acquisition or expression of the CPP response to food or morphine [88]. However, BP-897 alone produced CPA, a finding congruent with that of Gyertyán and Gál [121], and may also have anxiolytic properties [246].

A recent paper by Campiani et al. [44] reported the pharmacological evaluation of a series of novel arylalkylpiperazine structures related to BP-897 as well as BP-897 itself. BP-897 (1 mg/kg) significantly reduced the number of active lever presses by male Sprague–Dawley rats following re-exposure to cocaine-associated stimuli. In contrast, *compound 5q* (*N*-[4-[4-(2,4-dichlorophenyl)piperazin-1-yl]butyl]-5-chloroindole-2-carboxamide), a selective DA D_3 receptor partial agonist (K_i D_2 > 10000 nM; K_i D_3 = 0.38 ± 0.005 nM; K_i 5-HT_{1A} > 10000 nM) did not significantly alter cocaine-seeking behavior produced by environmental cues previously associated with cocaine self-administration. The explanation for the difference in the behavioral effects between these compounds remains to be elucidated.

It has been hypothesized that BP-897 exerts its anti-addictive actions via a selective partial agonism at D_3 receptors. However, antagonism at D_3 receptors and/or activity at other receptors might also explain some of the in vivo data. In support of the first argument (partial agonism vs. antagonism at D_3 receptors) are pharmacological data (forskolin-induced increase in cAMP) indicating that in NG 108-15 cells expressing human D_3 receptors, BP-897 behaves as a partial agonist compared to DA (59%) [224]. Similarly, in the mitogenesis assay, the maximum response elicited by BP-897 was 55% of that elicited by the full agonist quinpirole [23,224]. In cells expressing D_2 receptors, BP-897 alone does not inhibit cAMP accumulation or elicit mitogenesis [224]. However, it reversibly antagonizes quinpirole-induced mitogenesis, but only at concentrations significantly greater than those required for D_3 receptor stimulation [224]. Thus, based on the results obtained by Pilla et al. [224], BP-897 can be classified as a partial D_3 receptor agonist. However, recent evidence from studies using microphysiometry shows that in CHO-K1 cells transfected with human D_2 and D_3 receptors, BP-897 behaves as a full antagonist at both DA D_2 (pK_b = 8.05) and D_3 (pK_b = 9.43) receptors [305]. In addition, in CHO cells transfected with human D_3 receptors, BP-897 does not stimulate D_3 receptors and displays antagonistic effects in a [^{35}S]-GTPγS binding assay in cells expressing human D_3 receptors [302]. Clearly, different results in the intrinsic activity of partial agonists can be obtained using different assay systems. The observed

Notes to Table 1:
[a] D_2:D_3 K_i ratios are shown for different radioligands.
[b] See Ref. [11].
[c] See Ref. [11].
[d] See Ref. [224].
[e] See Ref. [11].
[f] See Ref. [11].
[g] See Ref. [276].
[h] See Ref. [11].
[i] See Ref. [11].
[j] See Ref. [11].
[k] See Ref. [11].
[l] See Ref. [229].
[m] See Ref. [186].
[n] See Refs. [236,313].
[o] See Ref. [201].
[p] See Ref. [23].
[q] See Ref. [291].
[r] See Ref. [216].

Table 2
Effect of mixed D_2/D_3 receptor antagonists in animal models of drug addiction[a]

Compound name	D_2:D_3 selectivity	Paradigm	Dose (mg/kg) and route of administration	Main finding	Reference
DS-121	3–5	Cocaine-induced locomotor activity in cocaine tolerant rats	0–7 (ip)	Potentiation	[91]
		Spontaneous locomotor activity	3.3–13.3 (sc)	Increase	[153]
			Intra-NAc (0.05–53 μg/side)	No effect	
			Intra-VTA (0.05–53 μg/side)	No effect	
			ICV bilateral (66.3 μg/side)	Increase	
		CPP	3.3–13.3 (sc)	Place preference	[153]
		BSR	3.3–13.3 (sc)	No effect	[153]
		Discriminative stimulus properties of amphetamine (0.5 mg/kg) and cocaine (5 mg/kg)	3.5–14 (sc)	Increase in amphetamine-like and cocaine-like responding	[58]
		Cocaine self-administration	3–10 (sc)	Decrease	[238]
		Progressive ratio breakpoint for cocaine self-administration	15 (ip)	Decrease	[154]
		Extracellular levels of DA in dorsal striatum	15 (ip)	Increase	[154]
				Potentiation of cocaine-induced increase in striatal DA levels	
Nafadotride	6–9	Development of amphetamine sensitization	25 μg/kg (sc)	Decrease	[230]
		Expression of cocaine sensitization	0.4 (ip)	Potentiation	[99]
		Cocaine self-administration under FR-5	1–3 (sc)	Increase	[43]
		Cue-induced cocaine-seeking behavior	1 (ip)	Decrease	[299]
		Apomorphine- and 7-OH-DPAT-induced reinstatement of food-seeking behavior	1 (ip)	Decrease	[89]
				No effect on food-primed food seeking	
		Sensitization-induced facilitation of appetitive conditioning	Intra-amygdala (20 μmol/ml; 0.5 μl/side)	Retardation of conditioned response	[221]
		Isolation rearing-induced facilitation of Pavlovian learning	Intra-amygdala (20 μmol/ml; 0.5 μl/side)	Abolition of acquisition of Pavlovian-conditioned approach	[222]
		Spontaneous locomotor activity	0.1–1 (sc)	Increase	[248]
			0.75–3 (ip)	Increase	[70]
		Morphine (10 mg/kg)-induced hyperactivity	0.3–1 (sc)	Blockade at 1 mg/kg	[61]
		MK-801-induced hyperactivity	0.3 and 1 (ip)	Blockade	[174]
		Climbing behavior in mice	0.1–1 (sc)	Increase	[248]
		Conditioned reaction time	0.1, 0.3, and 1 (sc)	Increased number of delayed responses at 1 mg/kg	[271]
		Catalepsy		AD_{50} = 16.4 (sc)[b]	[11]
		DA turnover in dorsal striatum		AD_{50} = 0.5 (sc)[c]	[11]
		Plasma prolactin levels		MED = 0.16 (sc)[d]	[11]
U99194	10–15	Social interaction	20 and 40 (sc)	Increase	[244]
		Spontaneous locomotor activity and rearing	5 and 10 (sc)	Increase	[108]
			20 and 40 (sc)	Decrease	[49,244, 249,298]

(continued on next page)

Table 2 (*continued*)

Compound name	D_2:D_3 selectivity	Paradigm	Dose (mg/kg) and route of administration	Main finding	Reference
		Spontaneous and morphine-induced locomotor activity	20 (ip)	Increase and blockade of morphine (20 mg/kg)-induced hyperactivity	[188]
		Development of amphetamine sensitization	ICV (20 μg × 7 days)	Decrease	[54]
		Discriminative stimulus properties of 7-OH-DPAT (0.01–1 mg/kg) or PD128907 (0.03 mg/kg)	2.5–20 (sc)	No effect/partial blockade	[57,105]
				Potentiation	[74]
		Amphetamine-induced enhancement of BSR	5–20 (sc)	Increase	[49]
		Discriminative stimulus properties of amphetamine (1 mg/kg) and cocaine (5 mg/kg)	10–40 (sc)	No effect	[18,19]
		Cocaine CPP	12 and 24 (sc)	No effect Place preference per se	[121]
		Ethanol CPP	10 and 20 (ip)	No effect Enhancement	[86] [31,32]
		Oral ethanol self-administration	10 and 20 (ip)	No effect	[31,32]
		Amphetamine (2.5 mg/kg)-induced contralateral rotation in 6-OHDA-lesioned rats	5, 10, and 20 (sc)	>98% DA depletion (ipsilateral rotations): no effect 80–97% DA depletion (contralateral rotations): blockade	[241]
		Drug discrimination	5–35 (sc)	Generalization to UH-232, scopolamine, trihexyphenidyl, and clozapine	[115]
		Catalepsy		AD_{50} > 40 (sc)[e]	[11]
		DA turnover in dorsal striatum		AD_{50} = 6.9 (sc)[f]	[11]
		Plasma prolactin levels		MED = 40 (sc)[g]	[11]
S14297	23–61	Catalepsy		AD_{50} > 20 (sc)[h]	[11,113,199]
		Plasma prolactin levels		MED > 40 (sc)[i]	[11]
		Discriminative stimulus properties of U99194	3–8 (sc)	Partial substitution (66%)	[17]
		Spontaneous locomotor activity	ED_{50} = 15.4 (ip) ED_{50} > 30 (sc)	Decrease No effect	[62]
PD152255	40–50	Amphetamine-induced locomotor activity	1, 3, and 10 (ip)	Decrease	
			1, 3, and 10 (sc)	No effect	[62]
		Discriminative stimulus properties of U99194	1–3 (ip) Suppression of responding at higher doses	No generalization	[17]

[a] For a detailed ethologically based approach comparing the ethograms of U99194A, GR103691, and nafadotride, the reader is referred to Ref. [59].
[b] AD_{50} was defined as the dose required for the induction of a half-maximal response (equivalent to 15 s).
[c] AD_{50} was defined as an increase in DOPAC:DA ratios to 150% relative to vehicle values.
[d] MED was defined as the lowest dose significantly different ($P < 0.05$) from vehicle control values.
[e] AD_{50} was defined as the dose required for the induction of a half-maximal response (equivalent to 15 s).
[f] AD_{50} was defined as an increase in DOPAC:DA ratios to 150% relative to vehicle values.
[g] MED was defined as the lowest dose significantly different ($P < 0.05$) from vehicle control values.
[h] AD_{50} was defined as the dose required for the induction of a half-maximal response (equivalent to 15 s).
[i] MED was defined as the lowest dose significantly different ($P < 0.05$) from vehicle control values.

efficacy of partial agonists could be related to differences in the level of receptor reserve and efficiency of functional coupling. Wood et al. [305] identified BHT920 as a full agonist, but the same compound was identified as only a partial agonist by Pilla et al. [224]. There are also distinct differences between the microphysiometry and mitogenesis assays that are worth noting. The mitogenesis assay requires sustained activation that can be complicated by desensitization and stability of the agonists. The microphysiometry assay involves measurements carried out in real time. The response observed in the microphysiometry assay is likely a direct consequence of activation of D_3 receptors whereas the

response seen in the mitogenesis assay represents an adaptation of the cell to continuous receptor activation and may be complicated by the presence of endogenous receptors and regulators. Finally, in anesthetized rats, in vivo extracellular recording demonstrated that iv administration of BP-897 (maximum dose of 8.2 mg/kg) did not significantly alter the firing rate of spontaneously active substantia nigra pars compacta (A9) DA neurons, but did antagonize quinpirole-induced inhibition of firing of these neurons with an ED_{50} of 1.1 mg/kg [302]. Taken together, these results suggest that BP-897 may also exert antagonist actions at the DA D_3 receptor and that one cannot exclude the possibility that BP-897's antagonism of quinpirole's action is partly related to antagonism at DA D_2 receptors.

In support of the second argument (cross-selectivity profile) are data showing that BP-897 has affinity for other neurotransmitter receptors (for a detailed review on BP-897, the reader is referred to Ref. [103]). BP-897 shows moderate affinity for α_1- and α_2-adrenergic receptors (K_i = 60 and 83 nM, respectively), 5-HT_{1A} (K_i = 84 nM), and DA D_2 receptors (K_i = 61 nM) [224] (see also Ref. [44]). BP-897 also displays potent antagonistic action at 5-HT_{2A} and α_1-adrenergic receptors (M.J. Millan, personal communication). The interaction with 5-HT_{2A} receptors may be important as a recent study in rats indicates that the 5-HT_{2A} receptor antagonist M100,907 attenuates the ability of a priming injection of cocaine to reinstate lever pressing [101]. Thus, the effect of BP-897 in some animal models of addiction could be partially related to its antagonist action at 5-HT_{2A} receptors. Finally, a recent study reported that the K_i of BP-897 for human D_4 receptors is 39 nM and the human D_4/D_3 ratio is 28 [23].

Altogether, both in vitro and in vivo data suggest that BP-897 may act as a D_3 receptor agonist or antagonist. In addition, one cannot rule out the possibility that part of BP-897's action is mediated by interaction with other receptors.

6. Role of DA D_3 receptors in drug addiction: studies with mixed DA D_2/D_3 receptor antagonists

A number of compounds were originally reported to be selective D_3 receptor antagonists, including (+)-AJ-76 [273,274], (+)-UH-232 [273,274], U99194A [122], nafadotride [248], GR103691 [206], and DS-121 [153]. However, evidence from a variety of studies indicates that these compounds either lack sufficient in vitro and/or in vivo selectivity or interact with other receptors and therefore cannot be characterized as selective D_3 receptor antagonists (see Table 1). For example, the in vitro selectivity of (+)-UH-232, (+)-AJ-76, nafadotride, and U99194A for D_3/D_2 is only 4–8, 2–6, 6–9, and 10–20-fold, respectively [118,175,272,298,307]. Functional and/or behavioral studies suggest that the aforementioned pharmacological agents produce effects associated with D_2 receptor antagonism. For example, systemic administration of (+)-UH-232, (+)-AJ-76, and nafadotride can induce catalepsy and increase prolactin levels in rats [11]. The acute administration of U-99194A, nafadotride, and (+)-UH-232 significantly increases DA turnover in the striatum, NAc, frontal cortex and olfactory tubercle [11]. Levant and Vansell [178] also examined the in vivo occupancy of D_2 receptors by nafadotride (0.1–10 mg/kg ip) in the EEDQ assay. Their results indicate that D_2 receptor antagonism contributes to the pharmacological actions of nafadotride at sc doses above 1 mg/kg and ip doses above 3 mg/kg. A detailed summary of the main behavioral pharmacology of DS-121, nafadotride, U99194, S14297, and PD152255 can be found in Table 2.

7. Role of DA D_3 receptors in drug addiction: studies with selective DA D_3 receptor antagonists—SB-277011-A

Radioligand binding studies have shown that SB-277011-A is a selective DA D_3 receptor antagonist with

Table 3
Effects of SB-277011-A alone in various pharmacology models[a]

Experimental paradigm	Doses	Main finding
Spontaneous locomotor activity	0–42.3 mg/kg po	No effect
Amphetamine-induced locomotor activity	0–51.3 mg/kg po	No effect
Phencyclidine-induced locomotor activity	0–51.3 mg/kg po	No effect
Apomorphine-induced climbing in mice	0–42 mg/kg po	No effect
Quinelorane-induced locomotor hypoactivity	0–41 mg/kg po	No effect
Quinelorane-induced reversal of amphetamine hyperactivity	0–41 mg/kg po	No effect
Quinpirole-induced deficit in prepulse inhibition	0–41 mg/kg po	No effect
Apomorphine-induced deficit in prepulse inhibition	0–41 mg/kg po	No effect
Differential reinforcement of low response rates	0–27.5 mg/kg po	No effect
Catalepsy	0–78.8 mg/kg po	No effect
Haloperidol-induced catalepsy	0–41 mg/kg po	No effect
Serum prolactin levels	0–93 mg/kg po	No effect
Rotarod performance	0–91.8 mg/kg po	No effect, except 91.8 mg/kg dose producing impairment in performance
Maximal electroshock seizure threshold	0–92 mg/kg po	No effect
Mouse Irwin profile	0–91.8 mg/kg po	No effect, except 91.8 mg/kg dose producing weak sedative-like effect
Delayed nonmatching test	0–41 mg/kg po	No effect

[a] See Ref. [229].

high affinity for the human (pK_i 7.95) and rat (pK_i 7.97) cloned DA D_3 receptor. The ratio of the in vitro D_3/D_2 affinity of SB-277011-A for human and rat is 120 and 80, respectively [229]. SB-277011-A has a 100-fold selectivity over 66 other receptors, enzymes, and ion channels [229]. SB-277011-A is a potent and competitive antagonist, with pK_b 8.4 (4 nM) in the microphysiometry in vitro functional assay using human cloned DA D_3 receptors expressed in CHO cells, and it maintains selectivity with respect to DA D_2 receptors (pK_b 6.5). SB-277011-A has also been shown to readily penetrate the rat brain with a steady-state brain/plasma ratio of 3.6:1 [229,283]. In the rat, SB-277011-A has an oral bioavailability of 43%, shows low clearance, and a half-life of 2.0 h. The drug appears to be metabolized by the liver enzyme hepatic aldehyde oxidase [16].

The effects of SB-277011-A per se in in vivo behavioral models are summarized in Table 3. SB-277011-A is devoid of typical DA D_2/D_3 receptor antagonist effects and does not show proconvulsant activity. Only very high doses of SB-277011-A (above 90 mg/kg po), which are significantly higher than those showing efficacy in models of addiction, produce weak sedative-like actions in the mouse Irwin test and impair performance in the rat rotarod test. In vivo brain microdialysis data indicate that administration of SB-277011-A (2.8 mg/kg po) reverses the decrease in extracellular DA levels produced by quinelorane (D_2/D_3 agonist) in the NAc, but not the dorsal striatum, a regional selectivity consistent with the distribution of DA D_3 receptors in the rat brain [229]. A single ip administration of 10 mg/kg of SB-277011-A significantly increases extracellular levels of DA, norepinephrine (NE), and acetylcholine (ACh) in the anterior cingulate cortex in freely moving rats [160]. It should be pointed out that SB-277011-A does not produce a functional antagonism of D_2 receptors in vivo as, unlike D_2 receptor antagonists, its systemic administration in rats does not (1) elicit catalepsy at doses that exhibit anti-addiction action [229,295]; (2) produce a rightward shift in brain stimulation reward curve [229]; (3) increase plasma prolactin levels [295]; (4) inhibit spontaneous or stimulant-induced locomotion [295]; (5) antagonize the action of quinpirole in the dorsal striatum, a brain region with a high density of D_2 receptors [295]; (6) increase DA levels in the striatum [295]; and (7) increase cocaine self-administration under a schedule of continuous reinforcement ([84,102,295]; see Section 7.4).

The following subsections will summarize the effects of SB-277011-A in animal models of drug addiction (see also Table 4).

7.1. Effect of SB-277011-A on nicotine self-administration and nicotine-triggered relapse to nicotine-seeking behavior

Andreoli et al. [6] examined the effect of SB-277011-A on self-administration of nicotine (0.03 mg/kg) using an FR-1/FR-2 schedule in male Wistar rats. SB-277011-A (3 or 10 mg/kg ip) did not affect the number of infusions/h or the number of active lever presses/h compared with the vehicle group at any dose tested. SB-277011-A also failed to modify the number of inactive lever presses/h. The effect of SB-277011-A was also examined on noncontingent nicotine-triggered (0.15 mg/kg) reinstatement of extinguished responding on an operant lever, the depression of which previously resulted in iv nicotine infusions, 24 h after cessation of the self-administration of nicotine [6]. While acute administration of nicotine (0.15 mg/kg sc) produced a significant increase in nicotine-paired lever

Table 4
Effect of SB-277011-A in animal models of drug addiction

Behavioral paradigm	Doses	Main finding	Reference
Nicotine self-administration (FR-1/FR-2)	3–10 mg/kg ip	No effect	[6]
Nicotine-triggered relapse to nicotine seeking	3–10 mg/kg ip	Blockade	[6]
Nicotine-induced enhancement of brain stimulation reward	3, 6, and 12 mg/kg ip	Blockade	[111]
Nicotine-conditioned locomotor activity	10 mg/kg ip	Blockade	[169]
Cocaine self-administration (FR-1)	3, 6, and 12 mg/kg ip	No effect	[295]
	5 and 20 mg/kg po	No effect	[102]
Cocaine-induced enhancement of brain stimulation reward	3, 6, and 12 mg/kg ip	Blockade	[295]
Acquisition of cocaine-induced conditioned place preference	0.3, 1, 3, and 10 mg/kg ip	Blockade	[295]
Expression of cocaine-induced conditioned place preference	0.3, 1, 3, and 10 mg/kg ip	Blockade	[295]
Expression of food-induced conditioned place preference	10 mg/kg ip	No effect	[295]
Cocaine-triggered relapse to cocaine seeking	3, 6, and 12 mg/kg ip	Blockade	[295]
Cue-triggered relapse to cocaine seeking	3, 10, and 30 mg/kg ip	Blockade	[51]
Cue-controlled cocaine seeking (2nd order reinforcement schedule)	0.3, 1, 3, 10, 20, and 30 mg/kg ip	Blockade	[84]
Sucrose self-administration under 2nd order reinforcement schedule	0.3, 1, 3, 10, 20, and 30 mg/kg ip	No effect	[84]
Cocaine self-administration under FR-10 and PR schedules of reinforcement	3, 6, 12, and 24 mg/kg ip	Blockade	[111]
Stress-induced relapse to cocaine seeking	3, 6, and 12 mg/kg ip	Blockade	[310]
	1.5 μg/0.5 μl/side NAc	Blockade	
	1.5 μg/0.5 μl/side dorsal Striatum	No effect	
Ethanol intake in ethanol-preferring vs. nonpreferring rats	3, 10, and 30 mg/kg ip	Blockade	[235]
Oral ethanol self-administration	10, 20, and 30 mg/kg ip	Blockade	[7]
Relapse to ethanol seeking	10, 20, and 30 mg/kg ip	Blockade	[189]
Expression of heroin-induced conditioned place preference	10 mg/kg ip	Blockade	[10]

presses in the vehicle/nicotine group compared with the vehicle/saline control group, SB-277011-A (3 and 10 mg/kg ip) produced a significant reduction in nicotine-paired lever presses compared to the vehicle/nicotine group. No significant changes in the number of inactive lever presses were seen during the noncontingent nicotine priming component of the experiment, suggesting a specific effect of SB-277011-A on drug-triggered drug-seeking behavior. These data suggest that SB-277011-A attenuates nicotine-triggered reinstatement of nicotine-seeking behavior without affecting stable maintenance of nicotine self-administration per se.

7.2. Effect of SB-277011-A on nicotine- and cocaine-induced enhancement of brain stimulation reward

The effects of SB-277011-A on nicotine-induced enhancement of BSR in male Long–Evans rats were also examined [45]. Rats were trained to lever press for BSR of the medial forebrain bundle at the level of the lateral hypothalamus and tested on a rate–frequency curve-shift brain reward paradigm. Administration of nicotine (0.5 mg/kg ip) robustly shifted BSR curves to the left, lowering brain reward thresholds by approximately 25%. Acute administration of SB-277011-A (3 and 6 mg/kg ip), given 1 h prior to BSR testing, failed to alter nicotine-enhanced BSR. However, 12 mg/kg of SB-277011-A significantly attenuated (70%) nicotine-induced enhancement of BSR. These data suggest that DA D_3 receptors play an important role in mediating nicotine-enhanced brain reward.

Administration of SB-277011-A (3 mg/kg ip), given 30 min prior to BSR testing, also completely blocked the robust enhancement of BSR produced by cocaine (2 mg/kg ip) in rats [295]. This effect cannot be attributed to a D_3 receptor antagonist-induced diminution of brain reward, as SB-277011-A alone did not significantly alter BSR thresholds at doses up to 12 mg/kg.

7.3. Effect of SB-277011-A on acquisition and expression of cocaine-induced conditioned place preference (CPP)

Acute systemic administration of SB-277011-A at all doses tested (0.3, 1, 3, and 10 mg/kg), 30 min prior to each administration of cocaine during the CPP acquisition phase, produced a significant blockade of the acquisition of cocaine-induced CPP [295]. This finding cannot be attributed to a D_3-antagonist-induced place aversion, as SB-277011-A by itself produced neither a significant place preference nor a significant place aversion at doses up to 10 mg/kg. The finding that SB-277011-A by itself produces neither preference nor aversion was recently confirmed using oral doses of SB-277011-A (5 and 20 mg/kg po) [121]. A single injection of 1, 3, or 10 mg/kg ip of SB-277011-A, 30 min prior to behavioral testing, also produced a significant blockade of the expression of cocaine-induced CPP [295]. Finally, daily ip administration of SB-277011-A (3 mg/kg) for 14 days prior to testing for expression of cocaine-induced CPP produced a robust blockade of the expression of cocaine-induced CPP [295]. It should also be noted that acute administration of SB-277011-A (10 mg/kg ip) did not block the expression of food-induced CPP [295].

In contrast to our CPP findings with SB-277011-A [295], Gyertyán and Gál [121] have reported that administration of 5 or 20 mg/kg po of SB-277011-A (30 min prior to cocaine administration) did not significantly alter the acquisition of a CPP response to cocaine (10 mg/kg ip) in male Sprague–Dawley rats. The reason for the difference between our findings and those of Gyertán and Gál [121] is unknown, although there were significant differences in methodology including (1) preconditioning the animals to the CPP apparatus vs. no preconditioning; (2) time period of 4 h between the administration of saline and cocaine vs. 24 h; (3) SB-277011-A administered via the po vs. ip route; and (4) SB-277011-A suspended in 5% Tween 80 vs. 2% methylcellulose.

7.4. Effect of SB-277011-A on cocaine self-administration and cocaine-triggered relapse to cocaine-seeking behavior

Acute administration of SB-277011-A (3, 10, and 12 mg/kg ip) did not affect stable maintenance of cocaine self-administration under a continuous reinforcement schedule [84,295]. These findings were also recently confirmed by Gál and Gyertyán [102] who showed that acute administration of SB-277011-A (5 and 20 mg/kg po) does not affect cocaine self-administration under continuous reinforcement. In contrast, the same study showed that both the DA D_1 receptor antagonist SCH-23390 (0.1 and 0.2 mg/kg ip) and the DA D_2-preferring receptor antagonist haloperidol (0.2 and 0.5 mg/kg po) produced a compensatory increase in lever pressing, of the type classically known to be produced by DA antagonists [110,311]. Finally, the mixed D_2/D_3 agonists PD-128907 (1 mg/kg sc) and 7-OH-DPAT (0.1 and 0.5 mg/kg sc) produced a significant decrease in lever pressing for cocaine. Interestingly, the same study showed that BP-897 (1 mg/kg ip) significantly increased cocaine self-administration under a continuous reinforcement schedule, suggesting that this effect of BP-897 at a dose of 1 mg/kg is unlikely to be mediated through an action at DA D_3 receptors. In addition to the findings of Gál and Gyertyán [102], it has been shown that cocaine self-administration is increased in a manner suggestive of a reduction in the reinforcing effects of the drug following administration of the nonselective D_2/D_3 or D_1/D_2 receptor antagonists YM-09-151-2 [37,279], spiperone [62,137], sulpiride, metoclopramide, thioridiazine, chlorpromazine, haloperidol, pimozide, or alpha-flupentixol [238,239].

Although SB-277011-A did not alter the self-administration of cocaine or nicotine in animals under FR-1

reinforcement, we have seen that it does attenuate the CPP and enhanced BSR produced by cocaine and nicotine. There is evidence suggesting that the nigrostriatal dopaminergic system plays an important role in habit formation, allowing animals to acquire and maintain performance [142]. Since, as previously mentioned, the nigrostriatal system is virtually devoid of D_3 receptors, it is possible that the inability of SB-277011-A to affect the self-administration of cocaine and nicotine is related to the relative lack of D_3 receptors in the nigrostriatal system. However, this hypothesis remains to be tested. An alternative hypothesis, supported by our findings of inhibition of cocaine self-administration by SB-277011-A when the cocaine reinforcement schedule is changed from FR-1 to higher FR ratios (see below), is that drug reinforcement on an FR-1 ratio constitutes too powerful a reinforcer for D_3 receptor antagonism to overcome.

A single noncontingent iv injection of 1 mg/kg cocaine produced robust reinstatement of extinguished operant behavior previously reinforced by iv cocaine injections. Acute pretreatment with SB-277011-A produced a dose-dependent attenuation of this cocaine-triggered reinstatement of extinguished cocaine-seeking behavior [295]. Importantly, SB-277011-A (3, 6, or 12 mg/kg) alone did not trigger reinstatement of cocaine seeking. Finally, over the dose range tested, SB-277011-A did not affect responses on the inactive lever.

7.5. Effect of SB-277011-A on cue-controlled cocaine-seeking behavior

The effect of SB-277011-A was also tested against cocaine-seeking behavior using second-order schedules of cocaine reinforcement, which provide an animal model of cue-controlled drug-seeking both prior to and after cocaine has been self-administered [84]. SB-277011-A (0.3, 3, 10, 20, and 30 mg/kg ip) produced a dose-dependent decrease in cocaine-seeking behavior maintained by a cocaine-associated conditioned reinforcer in both the first (drug-free) test interval and also following self-administration of cocaine (second interval) [84]. At higher doses, SB-277011-A also increased the latency to receive the first conditioned stimulus (CS) presentation and cocaine infusion, thereby decreasing the number of cocaine infusions self-administered under the second-order schedule of reinforcement. The decreased responding during the first and second intervals produced by pretreatment with SB-277011-A can be explained as an attenuation of the impact of the conditioned reinforcing properties of the drug-paired stimulus. Furthermore, the increase in latency to the first presentation of the contingent CS and the first cocaine infusion suggests that the decrease in cocaine intake under the second-order schedule is related to a decreased motivation to respond for cocaine. This suggestion is further strengthened by the observation that cocaine self-administration under an FR-1 schedule of reinforcement was not altered by SB-277011-A. Finally, the selectivity of D_3 receptors in mediating cue-controlled drug-seeking was further supported by the finding that SB-277011-A had no effect on responding for sucrose under similar second-order reinforcement.

Similar findings were reported in a model using male Sprague–Dawley rats trained to self-administer cocaine, while simultaneously establishing discriminative stimuli associated with, and predictive of, cocaine availability or nonavailability [51]. When given in doses ranging from 3 to 30 mg/kg ip, SB-277011-A decreased responding produced by re-introduction of cocaine-associated cues in a dose-dependent manner.

7.6. Effect of SB-277011-A on cocaine self-administration as determined by varying fixed-ratio and progressive-ratio reinforcement

The effect of SB-277011-A on cocaine self-administration under both fixed-ratio (FR) and progressive-ratio (PR) schedules of reinforcement in male Long–Evans rats was also examined [111]. The administration of SB-277011-A (3–24 mg/kg ip) did not significantly alter cocaine self-administration (0.75 mg/kg/injection) reinforced under an FR-1 schedule. However, SB-277011-A (24 mg/kg ip) produced a significant decrease in cocaine self-administration when (1) the unit dose of cocaine was decreased from 0.75 to 0.125–0.5 mg/kg, or (2) the work demand for cocaine was increased from an FR1 to FR10 schedule. Under a PR reinforcement schedule, SB-277011-A (6–24 mg/kg) produced a significant dose-dependent lowering of the PR breakpoint for cocaine self-administration. Furthermore, the 24 mg/kg dose significantly shifted the cocaine (0.25–1 mg/kg) dose–response breakpoint curve to the right. Finally, when SB-277011-A was substituted for cocaine in an FR schedule, it did not maintain cocaine self-administration behavior. Overall, these results indicate that antagonism of D_3 receptors by SB-277011-A significantly inhibits acute cocaine-induced reinforcement in an FR schedule following a reduction in reinforcement potency or an increase in work requirement. Why SB-277011-A significantly attenuates cocaine self-administration when the unit dose of cocaine is decreased or the FR reinforcement schedule is increased from 1 to 10 remains unknown. Compared with the amount of cocaine administered in other paradigms such as BSR and CPP, animals readily self-administer a significantly larger amount of cocaine under a continuous FR1 schedule for a high unit dose of cocaine. Therefore, it is possible that the high cocaine intake might produce increases in extracellular DA which (by competitive inhibition produced by SB-277011-A binding to the D_3 receptor) are too large for SB-277011-A to overcome. This hypothesis is partially supported by a study indicating a positive correlation between the amount of cocaine self-administered and extracellular NAc DA levels [220], and by the fact that the affinity of the D_3 receptor for DA is greater than that of all other DA receptor subtypes. One may also suggest that, given the half-life of SB-277011-A (2 h) vs.

the half-life of cocaine (20–40 min), SB-277011-A attenuates the "rush" effects induced by cocaine, and also possibly the negative compensatory effects that quickly follow the acute positive effects. Finally, it should also be noted that the FR1 schedule of reinforcement is useful for exploring patterns of rate of drug intake. However, the use of an FR1 schedule is less appropriate to assess changes in the reinforcing effects of drugs of abuse. In fact, rate of drug self-administration may be insensitive to changes in reinforcing efficacy and, even if changes are observed, there is little theoretical basis for interpreting these changes. The FR approach was mainly designed to explore how behavior changed when the contingencies between stimulus and response were altered; these procedures were not designed to estimate the magnitude of a reinforcer. Thus, FR responding is the equivalent to the rate of consumption and therefore corresponds to the rate of drug intake. This rate, however, is an ambiguous measure of drug efficacy. Since the PR breakpoint is an index of the relative strength of a reinforcer independent of response rate [8,135, 136,247], the shift in PR breakpoint produced by SB-277011-A indicates that SB-277011-A decreases the reinforcing value of cocaine in rats. Together, we conclude that SB-277011-A not only inhibits cocaine-induced incentive motivation and reinstatement, but also attenuates cocaine's rewarding efficacy.

7.7. Effect of SB-277011-A on stress-triggered relapse to cocaine-seeking behavior

One of the factors that can lead to relapse to drug use is exposure to stress. It has been well established in both animals [3,38,92,165,259–263,266,284] and humans [157,267,268] that exposure to stressors can produce reinstatement of self-administration of addictive drugs and/or drug-seeking behavior. Furthermore, studies in rats have indicated that morphine or cocaine CPP can be reactivated by footshock stress following drug-free periods [184,185,296]. Stressors such as food deprivation [265] and induction of a stress-like state by corticotropin-releasing factor (CRF) administration [261] reinstate heroin seeking in rats. As noted above, we have shown that acute administration of SB-277011-A significantly attenuates cocaine-induced reinstatement of cocaine-seeking behavior [295]. More recently, we have examined the effect of SB-277011-A on stress-induced reinstatement of cocaine seeking [310]. Administration of SB-277011-A (3–12 mg/kg ip) produced a dose-dependent decrease in the reinstatement of cocaine-seeking behavior produced by footshock stress. Furthermore, SB-277011-A microinjected intracranially into the NAc bilaterally (1.5 μg/0.5 μl/side) completely blocked stress-induced reinstatement of cocaine seeking, but microinjections into the dorsal neostriatum failed to affect stress-triggered reinstatement. The SB-277011-A-induced attenuation of stress-induced relapse to cocaine-seeking would appear not to be the result of anxiolytic and/or analgesic action since (1) SB-277011-A is inactive in paradigms that are used as screens for anxiolytic agents, and (2) SB-277011-A, compared to vehicle-treated animals, did not significantly alter avoidance behaviors such as foot flicking, foot withdrawal, or jumping following the administration of intermittent footshock stimuli (Z.-X. Xi et al. unpublished observations; also see Ref. [260]).

7.8. Effect of SB-277011-A on ethanol self-administration and relapse to ethanol-seeking behavior

We have examined the effect of SB-277011-A on the intake of ethanol in ethanol-preferring (P) and non-ethanol-preferring (NP) rats [235]. A single administration of SB-277011-A (3 mg/kg po) did not significantly alter ethanol intake in P or NP rats compared to vehicle-treated rats. However, compared to vehicle-treated animals, a single po administration of 10 or 30 mg/kg of SB-277011-A significantly decreased ethanol intake in P rats, and a single po administration of 30 mg/kg of SB-277011-A significantly decreased ethanol intake in both P and NP rats.

We also examined the effect of a single ip injection of 10, 20, or 30 mg/kg of SB-277011-A, and its vehicle, on the number of oral ethanol reinforcements and ethanol intake in adult male C57BL/6N mice [7]. Acute administration of either 10 or 20 mg/kg ip of SB-277011-A did not significantly alter oral ethanol self-administration compared to vehicle-treated animals. However, a single administration of 30 mg/kg ip of SB-277011-A significantly decreased the number of reinforcements (by 71%) and the amount of ethanol consumed (by 72%) compared to vehicle-treated animals.

In contrast to our findings, the mixed D_2/D_3 receptor antagonist U99194 enhances ethanol CPP but does not affect oral alcohol self-administration in Swiss–Webster mice [31,32]. In contrast, U99194 fails to alter ethanol CPP in DBA/2J mice [86]. Moreover, Narita et al. [209] reported that in D_3 receptor knockout mice, physical dependence to ethanol is increased, although another study indicates that deleting the D_3 receptor in C57BL/6J mice does not significantly alter the rewarding effects of ethanol as assessed by operant ethanol self-administration [33]. These findings are in direct contrast to our data indicating that selective antagonism at D_3 receptors by SB-277011-A significantly decreases the intake of ethanol by rats and mice compared to animals treated with vehicle. The discrepant findings with D_3 receptor knockout studies might be explained by changes during the development of the genetically modified animal to compensate for the absence of the D_3 receptor. In support of this suggestion are findings that haloperidol-treated animals acquire ethanol CPP normally [234] whereas DA D_2 receptor knockout mice fail to acquire the CPP response [65]. These findings demonstrate that the behavioral effects produced by a receptor antagonist are not always compatible with those produced by genetically deleting the receptor at which the antagonist

acts. For a detailed review on the role of DA D_3 receptors in the addictive properties of ethanol, the reader is referred to Ref. [131].

Using a new model of relapse to ethanol-seeking behavior in mice that we have recently developed [189], we have found that noncontingent ethanol administration or ethanol-associated cues can robustly reinstate ethanol-seeking behavior in mice behaviorally extinguished from their previous ethanol self-administration behavior. Acute pretreatment with SB-277011-A (10, 20, and 30 mg/kg ip) produced a dose-dependent attenuation of reinstatement of extinguished ethanol-seeking behavior [189].

7.9. Effect of SB-277011-A on opiate-induced conditioned place preference (CPP)

We have recently shown that the administration of 10 mg/kg ip of SB-277011-A significantly attenuates the acquisition and expression of the CPP response to 1.5 mg/kg ip of heroin in adult male Sprague–Dawley rats [10]. As previously discussed, SB-277011-A alone does not produce place preference or aversion or shift the BSR curve, suggesting that the effect of SB-277011-A on heroin CPP cannot be related to SB-277011-A itself producing reward or aversion. In contrast, others report that the incentive motivating effect of morphine is significantly enhanced in D_3 receptor knockout mice ([210], but see discussion above).

7.10. Summary of effects of SB-277011-A on addictive drug action

The effects of SB-277011-A in several animal models of drug addiction are summarized in Table 4. Together, these findings indicate that SB-277011-A can reduce cocaine-, nicotine-, ethanol-, and heroin-seeking behaviors. These effects are most likely related to the selective antagonism of D_3 receptors, as SB-277011-A is a selective high affinity D_3 receptor antagonist. In addition, the effects of SB-277011-A are unlikely to result from (1) SB-277011-A producing aversive effects as it does not produce a dysphoric shift in the BSR curve and does not produce CPA, or (2) SB-277011-A producing a rewarding/reinforcing effect as it does not produce a significant left shift of the BSR curve, is not self-administered, and does not produce CPP. SB-277011-A completely blocked the acquisition and expression of the CPP to cocaine. Since the acquisition phase involves storage and encoding of contextual stimuli, and the expression phase involves retrieval of memories of contextual stimuli, it is possible that SB-277011-A may block CPP by interfering with various aspects of encoding and retrieving memories. However, SB-277011-A does not appear to alter memory (as measured using a delayed nonmatched position test, D. Jones and J.J. Hagan, personal communication). Furthermore, acute administration of SB-277011-A significantly increases ACh levels in the anterior cingulate cortex [160] and reverses scopolamine-induced memory deficits as assessed by the threechoice point water labyrinth test [163]. Both of these effects would be expected to improve rather than to interfere with memory. Finally, SB-277011-A does not produce catalepsy or significantly alter locomotor activity [229] at the doses used for the BSR, reinstatement, and CPP experiments, suggesting that the effects observed in these paradigms were not due to interference with normal locomotion/coordination.

The minimum effective dose of SB-277011-A to attenuate cocaine-induced CPP (0.3 mg/kg) was significantly lower than the minimum effective dose to attenuate cocaine-triggered reinstatement (6 mg/kg). This difference could be related to the fact that the reinstatement-triggering dose of cocaine (1 mg/kg iv) may have been significantly supra-threshold (see, e.g., Ref. [76]) and therefore more difficult to overcome. Another explanation could be that cocaine-induced CPP is fundamentally more sensitive to D_3 antagonism than cocaine-induced reinstatement. In this regard, the absence of SB-277011-A dose-dependence in blocking cocaine-induced CPP within the dose range used in the reported experiments may be relevant; dose dependence may exist at lower doses. Similarly, it is possible that cocaine-associated cue-induced increases in forebrain DA are substantially lower than cocaine-induced increases [35,109], and perhaps easier for D_3 antagonism to surmount.

The different experimental animal paradigms described in previous sections of the present review and summarized in Table 4 each have unique relevance for different aspects of human cocaine addiction. BSR presumably measures the direct rewarding properties of addictive drugs and may come closest to modeling the drug-induced subjective "high". CPP presumably measures drug-seeking behavior specifically evoked by the incentive salience [24,25,85, 138,285] acquired by environmental cues after repeated association with an addictive drug. Reinstatement presumably measures drug-seeking behavior specifically evoked by re-exposure to drugs, cues, or stressors after behavioral extinction and pharmacological detoxification. The present data suggest that selective DA D_3 antagonism may hold highest promise for attenuating cue-evoked relapse to addictive drug use. To date, few other potential pharmacotherapies have been found which block cue-triggered reinstatement (see, however, [75]). Therefore, a relatively unique therapeutic utility may exist for selective DA D_3 antagonists.

Our results are also the first to show that the acute systemic administration of a potent and highly selective brain penetrant D_3 receptor antagonist significantly decreases stress-induced reinstatement to iv drug-seeking behavior. We therefore suggest that D_3 receptors in the brain are involved in mediating/modulating stress-induced relapse. This suggestion is novel, as stress-triggered relapse has heretofore appeared to be predominantly mediated by noradrenergic and CRF neurotransmitter mechanisms (for reviews, see Refs. [223,262,264,266]). Importantly, how-

ever, a role for DA has not been ruled out. For example, acute stress rapidly activates DA neurons in the VTA [144] and increases DA release in the NAc [143,260,289] and mPFC [277,312]. Stress-induced elevation of NAc DA correlates temporally with reinstatement of heroin seeking [259]. Stress appears to stimulate NAc DA release by activating an excitatory projection from the mPFC to glutamate receptors on VTA DA neurons [203]. In addition, stress may also activate mesolimbic DA via CRF release in the midbrain and amygdala [262]. Intracerebroventricular infusion of CRF mimics stress-induced heroin seeking [261] and enhances DA release in the hypothalamus and mPFC [164], although CRF effects on DA release in the NAc have not been reported. Finally, it has been postulated that DA may play an indirect/modulatory role in footshock stress-induced reinstatement [262,266]. Overall, it is likely that multiple neurochemical and anatomical substrates are involved in stress-induced relapse. While our findings with NAc microinjections of SB-277011-A suggest that the NAc may be involved in stress-triggered relapse, there is an important caveat. The NAc is close to the BNST, a region implicated in stress-triggered relapse [262,266,284]. The latency between microinjections and testing in the experiments described in the present review appears sufficient for diffusion of SB-277011-A from NAc to BNST. As discussed earlier, the BNST is a brain area that has a moderate to high density of D_3 receptor mRNA, although this area seems to have few or no D_3 receptors as ascertained by radioligand binding studies. Thus, additional studies are needed to localize the intracerebral site of action for SB-277011-A's protective effects against stress-triggered reinstatement of drug-seeking behavior.

8. Role of DA D_3 receptors in drug addiction: further confirmation with similar and structurally diverse selective D_3 receptor antagonists

Confirmation that it is D_3 receptor blockade that is important in mediating the effects of SB-277011-A is provided by reports that structurally dissimilar D_3 receptor antagonists possess similar in vivo properties. Recent studies have shown that *trans*-3-(2-(4-((3-(3-(5-methyl-1,2,4-oxidiazolyl))phenyl)carboxamido)cyclohexyl)ethyl)-7-methylsulfonyl-2,3,4,5-tetrahydro-1*H*-3-benzapine (SB-414796), another potent and selective DA D_3 receptor antagonist, can also block the expression of cocaine-induced CPP [186]. Furthermore, the effect of the selective D_3 receptor antagonist NGB-2904 [236,313] has recently been examined in animal models of addiction [309]. It has been reported that, using baculovirus expression of rat DA receptors in vitro, the selectivity of NGB-2904 for D_3 vs. D_2 is 830 as determined by D_2 and D_3 receptor binding and use of the radioligand [^{125}I]-IABN [214]. NGB-2904 was also shown to be a functional antagonist in the mitogenesis assay using human D_3-transfected CHO cells [236]. It was found that in male Long–Evans rats, acute ip administration of NGB-2904 (1) dose-dependently (0.1, 1, or 5 mg/kg) attenuated iv cocaine self-administration maintained by an FR-2 schedule of reinforcement; (2) significantly reduced motivation for cocaine reward (1 or 5 mg/kg), manifested as a significant reduction in progressive-ratio breakpoint for iv cocaine self-administration; and (3) inhibited cocaine-triggered reinstatement of cocaine-seeking behavior in a dose-dependent manner [309]. The inhibitory effect of NGB-2904 on cocaine-taking and cocaine-seeking behavior was prolonged (2–3 days) after a single ip injection. This phenomenon may result from lipid sequestration of NGB-2904, which is highly lipophilic (clogD = 6.94). Importantly, NGB-2904 (1 mg/kg/infusion) cannot by itself maintain iv self-administration when substituted for cocaine. Finally, when tested over a broad dose range (0.1–10 mg/kg), NGB-2904 alone had no significant effects on rat locomotor behavior.

Together, the results obtained with the selective DA D_3 receptor antagonists SB-414796 [186], NGB-2904 [309], and *compound 5p* (*N*-[4-[4-(2,4-dichlorophenyl)piperazin-1-yl]butyl]indole-2-carboxamide) [44] all confirm our previous findings with SB-277011-A and further strengthen the hypothesis that central D_3 receptors play an important role in the rewarding and incentive motivating effects of cocaine.

Other compounds have been reported to exhibit high in vitro selectivity for D_3 receptors whose anti-addiction profiles remain to be characterized. As two previous excellent reviews by Crider and Scheideler [64] and by Hackling and Stark [123] have been published on the medicinal chemistry and selectivity of various compounds for the D_3 receptor, we will only discuss the profiles of some recently synthesized compounds that may be worth examining in models of addiction.

Using [^{3}H]-antagonist radioligands, it has been reported that 2-(3-[4-(2-*tert*-butyl-6-trifluoromethyl-pyrimidin-4-yl)-piperazin-1-yl]-propyl-sulfanyl)-^{3}H-pyrimidin-4-one fumarate (A-437203) has D_{2S}/D_3 and D_{2L}/D_3 receptor selectivity ratios of 45 and 120, respectively, using HEK293 cells transfected with human D_2 and D_3 receptors [291] (see also Ref. [53]). Functional studies in cellular systems transfected with human D_3 receptors indicate that A-437203 lacks intrinsic activity at the D_3 receptor but antagonizes agonist-induced actions with p*K* values of 9 and 7.5 [291]. This compound does not produce catalepsy at doses as high as 464 mg/kg po. Systemic administration of A-437203 induces c-*fos* expression in the NAc and islands of Calleja, an effect reported for other selective D_3 receptor antagonists, and blocks the quinpirole-induced decrease of extracellular DA in the mPFC and NAc [87]. Furthermore, chronic administration of A-437203 for 21 days produces a significant and selective decrease in the number of spontaneously active VTA DA neurons in anesthetized rats [85], a finding similar to that reported for SB-277011-A [9].

The compound 3aR, 9bS-*N*-[4-(8-cyano-1,3a,4,9b-tetrahydro-^{3}H-benzopyrano[3,4]pyrrole-2-yl)-butyl]-(4-phenyl)

benzamide (S33084) has been reported to be a selective D_3 receptor antagonist [67,68,90,200,201] with more than 200-fold selectivity compared to 40 other binding sites [200]. Binding studies using cloned human D_3 and D_2 receptors indicate that the D_2/D_3 receptor selectivity ratios using [^{125}I]-iodosulpiride and [^{3}H]-spiperone are 120 and 125, respectively [200]. S33084 also exhibits high selectivity for cloned and native rat D_3 receptors [68]. S33084 alone does not significantly modify [^{35}S]GTPγS binding at human D_3 receptors [200]. In addition, S33084 does not activate the ERK1 and ERK2 species of MAP kinase in CHO cells transfected with human D_3 receptors, but antagonizes the induction of MAP by DA [200]. The pA_2 value of S33084 to antagonize the stimulation of D_3 receptors by DA is 9.69. S33084 has low affinity for a number of other neurotransmitter receptors, including D_1 and D_4 receptors [200]. Behavioral studies in rats indicate that S33084 does not induce catalepsy, significantly alter locomotor responses to amphetamine or cocaine, or antagonize methylphenidate-induced gnawing [201]. Furthermore, S33084 does not increase prolactin secretion in rats [201]. Thus, these functional studies suggest that S33084 is not a D_2 receptor antagonist. Similarly, the compound 2(R,S)-(di-n-propylamino)-6-(4-methoxyphenylsufonyl methyl)-1,2,3,4-tetrahydronaphthalene (GR218,231) [207] is a selective hD_3 vs. hD_2 receptor antagonist with hD_2/hD_3 receptor selectivity ratios using [^{125}I]-sulpiride and [^{3}H]-spiperone of 60 and 100, respectively, and a neurochemical profile similar to that of S33084 [200,201].

A series of studies published by Austin and colleagues reports the synthesis of various novel compounds with excellent in vitro selectivity for D_3 vs. D_2 receptors. A novel substituted 1,2,3,4-tetrahydroisoquinoline with a 7-CF_3SO_2O substituent and a 3-indolylpropenamido group has a D_2/D_3 receptor selectivity ratio of 150 as determined in CHO cells transfected with human D_2 and D_3 receptors [13]. A 2,3,4,5-tetrahydro-1*H*-3-benzazepine derivative ("compound 20") has a 130-fold selectivity for D_3 vs. D_2 receptors [14]. In addition, a 5-substitutued-2,3-dihydro-1*H*-isoindole has at least 100-fold selectivity for D_3 receptors over other aminergic receptors [15].

Recently, it has been reported that the benzothiophenes designated FAUC346 and FAUC365 have high affinity for human D_3 receptors, with K_i values of 0.23 and 0.5 nM, respectively [23]. The hD_{2L}/hD_3 receptor selectivity ratios for FAUC346 and FAUC365 are 380 and 7200, respectively. In the mitogenesis assay in CHO cells transfected with hD_3 receptors, FAUC346 displayed partial agonist action whereas FAUC365 lacked intrinsic action, suggesting that it is a D_3 receptor antagonist. The compounds designated 1c, 3a, 3b, 3e, and 3f have hD_{2L}/hD_3 receptor selectivity ratios of 72, 100, 210, 93, and 170, respectively [23].

Wright et al. [306] reported the synthesis of benzimidazole derivatives, one of which, 3-[4[1-[4-[2-[4-(3-diethylamino-propoxy)phenyl]-benzoimidazol-1-yl-butyl]-1*H*-benzoimidazol-2-yl]-phenoxy]propyl]diethylamide (PD 58491), was found to be a selective D_3 receptor antagonist. A subsequent study, using CHO cells transfected with human D_3 and D_{2L} receptors, indicated that PD 58491 has a D_2/D_3 receptor selectivity ratio of 120 [300]. PD 58491 antagonized the quinpirole-induced stimulation of [3H]-thymidine uptake in CHOpro-5 cells but lacked significant intrinsic activity by itself [300]. PD 58491 only partially blocked the decrease in DA synthesis in the striatum and mesolimbic system of rats produced by the putative selective D_3 receptor agonist PD 128907. This finding may be related to stimulation of D_2 autoreceptors by PD 128907, and PD 58491 would not antagonize this action, a hypothesis congruent with studies suggesting that PD 128907 can activate D_2 receptors in the rat brain. It was also hypothesized that there may be a high presynaptic receptor reserve of D_3 receptors, although this must still be determined.

Park et al. [216] have reported that the compound 1-(2-ethoxy-phenyl)-4-[3-(3-thiphen-2[yl-isoxzaolin-5-yl)-propyl]-piperazine (KCH-1110) has an in vitro hD_3/hD_2 ratio of 90. However, it is likely that this compound is an antagonist at D_2 receptors in vivo as it significantly (1) decreases basal locomotor activity in mice at doses of 2.5 and 5 mg/kg; (2) antagonizes apomorphine-induced climbing in mice; (3) increases serum prolactin levels in rats at a dose of 10 mg/kg; (4) antagonizes 7-OH-DPAT-induced hypothermia in mice; and (5) induces catalepsy, albeit mild, at 30 mg/kg. These effects are generally indicative of D_2 receptor antagonism. In addition, these effects of KCH-1110 are not seen after the administration of high doses of SB-277011-A [229]. The authors did not examine the effect of KCH-1110 against drugs of abuse in animal models of addiction (e.g., self-administration, CPP, reinstatement). Interestingly, the pretreatment of mice with 1 mg/kg of KCH-1110 significantly attenuated cocaine-induced hyperlocomotion. However, there is no significant correlation between a compound's efficacy to increase locomotor activity and its abuse potential. Finally, it should be noted that Park et al. [216] did not report whether KCH-1110 interacted with other CNS receptors to any significant degree.

9. Conclusions

The two compounds that have been systematically tested in animal models of drug addiction are the partial DA D_3 receptor agonist BP-897 and the selective DA D_3 receptor antagonist SB-277011-A (see Table 4). The sites of action of SB-277011-A and BP-897 in the CNS remain to be elucidated. There is considerable evidence that DA plays an important role in the addictive properties of drugs of abuse (for reviews, see Refs. [22,34,96–98,104,138,171,237,242,303,304]). Indeed, virtually all abusable substances activate the mesolimbic DA system [46,47,79,139,140,220,240,288] (for reviews, see Refs. [104,155]). However, the concept that the mesolimbic DA system simply encodes hedonic tone has been called

into question. Analysis of response patterns of single DA neurons to reward presentation has led Schultz and colleagues to suggest that mesolimbic DA may be more involved in prediction of reward and the use of such information to strengthen behaviors and increase their future likelihood. Schultz and colleagues have suggested that the DA signal may constitute an alert message about reward prediction error that rapidly informs postsynaptic structures about unexpected rewards or reward omissions, without detailed information about the nature of the reward per se. The advantage of such a reward alert signal would be to allow rapid behavioral reactions towards rewarding stimuli, while the exact nature of the reward would be evaluated by slower systems during the approach behavior to the rewarding stimulus (for a recent review, see Ref. [251]). On the other hand, Wise and colleagues have argued compellingly for a crucial role for meso-accumbens DA in the actual mediation of reward—if not a final common path for all rewards, at least an intermediate common path for most rewards [303], and we [104] and others [34,46,79,139,140,220,288] have argued that elevation of brain DA constitutes a neurochemical substrate of the reward produced by addictive drugs (and by drug-associated environmental cues; see e.g., Refs. [82,109,141]), not merely a correlate.

The circuitry that mediates reinstatement of drug-seeking behavior is complex [4,5,26,116,118,131,177,211,218,270, 301,303], but key elements involve DA mechanisms. For example, the presentation of a drug-associated CS to animals can induce large conditioned increases in NAc DA [82,141], suggesting that DA in the NAc is involved in cue-controlled drug seeking [308] through interactions with limbic afferents to the NAc [81,83]. Cue incentive properties [24,242] appear mediated by hippocampal and amygdaloid mechanisms [55,152,196]. The amygdala plays an important role [94], particularly in drug-enhanced stimulus–reward associations [127,243] which may, in conjunction with enhanced stimulus–response associations [79], underlie drug craving and compulsive drug taking at the human level [80,215]. Lesions of the central amygdala (CeA) or infusion of 7-OH-DPAT into this brain area can attenuate the acquisition of a Pavlovian approach response [133,134, 217] and can impair the enhancement of instrumental behavior by presentation of a Pavlovian CS [125,126]. Interestingly, lesions of the basolateral amygdala (BLA) can impair the acquisition of cocaine seeking under a second-order schedule [301]. In addition, reversible inactivation of the BLA by lidocaine can block both the acquisition and expression of the response-maintaining properties of a CS under both second-order and reinstatement conditions [119,145,158]. Recent studies have also shown that direct microinfusion of D-amphetamine into the BLA can potentiate cue-triggered relapse to cocaine seeking in a dose-dependent manner without affecting extinction responding [166]. Furthermore, electrical or chemical stimulation of the BLA can reinstate cocaine-seeking behavior following behavioral extinction of the cocaine-seeking habit [129], as can electrical stimulation of the hippocampus, another brain structure implicated in the storage and retrieval of memories underlying the cue-incentive properties of addictive drugs [294]. Within the amygdala, direct infusion of the nonselective muscarinic receptor antagonist scopolamine into the BLA during the acquisition of conditioned pairing of a light/tone stimulus with cocaine self-administration can also disrupt cocaine-seeking behavior maintained by the cocaine-associated cues [255]. In contrast, the same scopolamine treatment given just prior to the reinstatement test fails to affect conditioned cue-induced relapse to cocaine seeking [255]. Reversible inactivation of the BLA, anterior cingulate cortex, or prelimbic cortex just prior to the reinstatement test impairs the ability of a light/tone stimulus to reinstate extinguished lever pressing for cocaine-paired stimuli [193]. These results are congruent with the finding that neural activity in the VTA, anterior cingulate cortex, NAc core, and ventral pallidum is necessary for cocaine-induced reinstatement of drug-seeking behavior [192]. It has also been shown that stress-induced relapse may be mediated by the BNST and CeA [93,173,264].

Such findings as those noted above point toward an important role of the BLA in stimulus–reward associations that mediate cue-triggered reinstatement of cocaine-seeking behavior. Enhanced monoaminergic tone in the BLA appears to increase the motivational properties or salience of cocaine-associated cues during reinstatement of cocaine-seeking behavior, whereas inactivation of the BLA produces the reverse effect. The CeA may mediate conditioned increases in DA measured in the NAc following the noncontingent presentation of a CS [82,141] perhaps via projections to the VTA [218] and seems to play a key role in stress-triggered relapse to cocaine-seeking behavior. Finally, the anterior cingulate cortex seems to serve as a common link in the neural circuitry underlying reinstatement of drug-seeking behaviors, perhaps because the anterior cingulate cortex is critically involved in the discrimination of multiple stimuli on the basis of their association with reward [48], in shifting away from spatial locations previously associated with reward (response perseveration), attention, and the ability to adequately plan actions involved in fear responding (for a complete review, see Ref. [130]). Thus, these results point towards the anterior cingulate cortex as a key component involved in the temporal patterning of behavioral sequences.

Given that SB-277011-A is effective in attenuating the action of a number of addictive drugs in various paradigms, one might hypothesize that (1) the cue-, drug-, and stress-induced reinstatement circuits may, in fact, have a common final pathway and that the efficacy of SB-277011-A is due to blockade of D_3 receptors in this pathway, and/or (2) D_3 receptors are located at critical junctions in the pathways involved in cue-, drug-, and stress-induced reinstatement. Indeed, DA D_3 receptors are present in moderate to high densities in the amygdala, NAc, VTA, BNST, and mPFC. In

a recent series of pharmacological magnetic resonance imaging (MRI) experiments, we have shown that pretreatment with SB-277011-A, at a dose behaviorally effective against drug-seeking, potentiates the rapid relative cerebral blood volume (rCBV) response to amphetamine in regions including, but extending beyond, the D_3-rich areas, while SB-277011-A itself produced only limited activation and gradual rCBV changes commensurate with its pharmacokinetics [254]. Importantly, the regions showing increased rCBV due to SB-277011-A alone did not correspond to the foci of dense D_3 receptor distribution and thus did not coincide with those foci showing a potentiated response to amphetamine. SB-277011-A itself produced focal bilateral activation in the entorhinal cortex, lateral globus pallidus, and CeA. The pattern of potentiation was more widespread and included the NAc, islands of Calleja, anterior cingulate and retrosplenial cortices, thalamus, dorsal striatum, BNST, and ventral subiculum. These findings suggest that the efficacy of selective DA D_3 receptor antagonists in attenuating reinstatement of drug-seeking behavior may in fact proceed via increased activity in both the extended amygdala and the mesolimbic DA system. Additional studies must be conducted in order to determine the specific brain areas involved in the effects of SB-277011-A in the CPP, BSR, and reinstatement paradigms. Studies examining the effect of SB-277011-A microinjections into areas such as the amygdala, VTA, NAc, and anterior cingulate cortex are warranted and have been initiated.

Acknowledgments

We thank Sharon Buie for assistance with manuscript preparation and Arlene C. Pak and Jeremy Gilbert for helpful comments, criticisms, and assistance with reference citations.

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Pharmacology & Therapeutics 90 (2001) 231–259

Pharmacology & Therapeutics

Associate editor: K.A. Neve

Dopamine D_3 receptor as a therapeutic target for antipsychotic and antiparkinsonian drugs

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Abstract

The cloning of the gene for the D_3 receptor and subsequent identification of its distribution in brain and pharmacology allowed for serious consideration of the possibility that it might be a target for drugs used to treat schizophrenia and Parkinson's disease (PD). That is because it is highly expressed in limbic regions of the brain, exhibits low expression in motor divisions, and has pharmacologic similarity to the D_2 receptor. Thus, antipsychotics that were presumed to block D_2 receptors also had high affinity for the D_3 receptor. Dopamine agonists used to treat the clinical symptoms of PD also have high affinity for the D_3 receptor, and two D_3 receptor-preferring agonists were found to be effective for treatment of PD. Many compounds achieving high potency and selectivity are now available, but few have reached clinical testing. Recent findings with respect to the anatomy of this receptor in human brain, altered expression in schizophrenia and PD, and biological models to study its function support the proposal that it is a target for development of drugs to alleviate symptoms in neuropsychiatric and neurologic disorders. Because of distinct aspects of regulation of the D_3 receptor, it represents a unique target for therapeutic intervention in schizophrenia without high potential for unintended side effects such as tardive dyskinesia. It may also be that D_3 receptor agonists can provide neuroprotective effects in PD and can modify clinical symptoms that D_2 receptor-preferring agonists cannot provide.

Keywords: Striatum; Caudate putamen; Nucleus accumbens; Islands of Calleja; Limbic

Abbreviations: BDNF, brain-derived neurotrophic factor; CN, caudate nucleus; CPu, caudate putamen; DA, dopamine; DAT, dopamine transporter; DYN, dynorphin; ENK, enkephalin; GPe, globus pallidus external segment; GPi, internal segment of the globus pallidus; 5-HT, serotonin; 7-OH-DPAT, 7-hydroxydipropylaminotetralin; ICj, Islands of Calleja; IEG, immediate early gene; L-dopa, levodopa; MPP+, *N*-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NA, nucleus accumbens; NAC, core of the nucleus accumbens; NAS, shell of the nucleus accumbens; 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; PET, positron emission tomography; PPX, pramipexole; PUT, putamen; SN, substantia nigra; SNpc, substantia nigra pars compacta; SNpr, substantia nigra pars reticulata; SPECT, single-photon emission computed tomography; STN, subthalamic nucleus; Sub P, substance P; TH, tyrosine hydroxylase; VP, ventral pallidum; VTA, ventral tegmental area; WT, wild-type.

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0163-7258/01/$ – see front matter
PII: S0163-7258(01)00139-5

1. Introduction

Dopamine (DA) plays an important, and often necessary, role in a wide variety of behaviors and functions, ranging from movement to emotion, sensitization to addiction, and development to plasticity. In part, this diversity reflects the organization of multiple types of DA receptors, the neurons that express those receptors, and their sources of DA innervation. It was not fully appreciated until the late 1970s and early 1980s that different behavioral functions could be mediated by the actions of DA at multiple DA receptors (Clark & White, 1987; Joyce, 1983; Kebabian & Calne, 1979). At that time, it was shown conclusively that the two biochemically identified DA receptors, D1 and D2, were both necessary for normal locomotor behavior. In the late 1980s and early 1990s, the cloning of the five identified subtypes of DA receptors, which are related to the D1 and D2 subfamilies, allowed for new insights into the wider diversity of the actions of DA. Three of the DA receptors, D_2, D_3, and D_4, belong to the D2 subfamily and couple to inhibitory G-proteins (Civelli et al., 1993). As all antipsychotics and antiparkinsonian agents act predominantly through the D2 subfamily of receptors, it is important to understand what functions the subtypes might mediate. This is particularly true since these receptor subtypes are differentially expressed in regions of the human brain, providing a rationale for how they could mediate different actions of DA (for a review, see Joyce & Meador-Woodruff, 1997). The functional significance of the D_4 receptor is questionable and the rationale for the development of D_4 receptor ligands has been disputed (Sanner, 1998). In contrast, a strong rationale can be made for the development of compounds that interact with the D_3 receptor because of its pharmacology, distribution in brain, and proposed role in neuropsychiatric and neurological disorders.

The D_3 DA receptor, a G-protein-coupled receptor with seven transmembrane segments, exhibits properties similar to that of the D_2 receptor, but has a distribution in the brain that is only partially overlapping with the D_2 receptor (Sokoloff et al., 1990). Drugs originally identified as having high affinity for the D_2 receptor exhibit similarly high affinity at the D_3 receptor radiolabeled with [^{125}I]iodosulpride or [^{3}H]spiroperidol (Audinot et al., 1998; Coldwell et al., 1999; Mierau et al., 1995; Newman-Tancredi et al., 1999; Perachon et al., 1999; Sautel et al., 1995b). Like the D_2 receptor, the D_3 receptor is known to inhibit adenylyl cyclases, but initially its functional activity was considered weaker than that of the D_2 and D_4 receptor in Chinese hamster ovary cells, a putatively responsive cell line (Chio et al., 1994; McAllister et al., 1995; Missale et al., 1998; Potenza et al., 1994). It was postulated that this could reflect weak coupling to G-proteins (Gardner et al., 1996; Malmberg et al., 1998). More recent studies with a variety of functional assays have identified efficient coupling of the D_3 receptor to $G_{\alpha i/o}$ subtypes of G-proteins (Coldwell et al., 1999; Newman-Tancredi et al., 1999; Vanhauwe et al., 1999; Zaworski et al., 1999) and inhibition of forskolin-stimulated adenylate cyclase (Perachon et al., 1999). Nonetheless, important questions remain as to what G-protein subtypes couple to the D_3 receptor in vivo and what other signaling pathways exist that have not been identified adequately (Cussac et al., 1999; Newman-Tancredi et al., 1999; Watts & Neve, 1997). Additionally, guanine nucleotides appear to have little effect on the binding of agonists to the D_3 receptor in vivo and in vitro (Burris et al., 1994; Gurevich et al., 1997; Mierau et al., 1995; Vanhauwe et al., 1999). This appears to allow the D_3 receptor to retain high affinity in vivo for DA (Levant, 1995; Zhang et al., 1999). How this might affect the ability of drugs to functionally interact with the D_3 receptor in vivo is open to speculation.

While the existence of the D_3 subtype of the D2 class of DA receptors is indisputable, due in large part to the historic deficiency of ligands with high selectivity for this subtype, the function of D_3 receptors has remained elusive. Nonetheless, recent evidence is surfacing that converges on some exciting common themes, some of which are directly relevant to two profoundly debilitating neuropathologies of the DA system, schizophrenia and Parkinson's disease (PD). Because of the pharmacological similarity of the D_2 and D_3 DA receptor, the D_3 receptor has to be considered a potential target for antipsychotic and anti-Parkinsonian drugs (Levant et al., 1999; Schwartz et al., 2000; Sokoloff et al., 1990). Relief of symptoms in PD is thought to occur as a consequence of activation of D2-like receptors (Loschmann et al., 1992; Nomoto et al., 1985; Vermeulen et al., 1999). DA agonists used in the treatment of PD (Table 1) have as high or higher affinity for the D_3 receptor, providing D_3 receptor preference. Thus, stimulation of D_3 receptors by antiparkinsonian drugs must be considered a therapeutic target (Fig. 1). In this review, we present an overview of the

Table 1
Drug affinities (nM) of agonists to cloned human DA receptors

Drug	hD_2 receptor			hD_3 receptor			hD_1 receptor	hD_4 receptor	Ratio[a]	Ratio[b]	Ratio	Ratio
	K_i	K_H	K_L	K_i	K_H	K_L	K_i	K_i	D_3/D_2	D_3/D_2	D_3/D_1	D_3/D_4
DA[c]	590	56	2300	18	3.2	76			0.03	0.06		
DA[d]		44	4265	55[e]						1.25		
DA[f]	700			40				70	0.06			0.57
Pergolide[c]	33	0.6	38	4[e]			310		0.12	67	0.013	
Pergolide[f]	8			1.6				1	0.2			1.6
Bromocriptine[c]	12[e]			12[e]			1500		1			
Bromocriptine[f]	3			2				20	0.67			
Bromocriptine[g]	5.3			7.4					1.39			
Lisuride[f]	0.2			0.2					1			
Lisuride[g]	1.4			1.7					1.21			
Pramepixole[c]	616	23	1600	8.5	0.7	15	> 50,000		0.14	0.03	0.0002	
Pramepixole[f]	1000			16				30	0.02			0.53
Pramepixole[h,i]		2.1	139		0.5	3		3	0.24			0.17
Talepixole[f]	1500			100				150	0.07			0.67
Talepixole[g]	390			33					0.08			
Ropinirole[c]	970	75	2000	61	22	140	> 50,000		0.06	0.29	0.0012	
Ropinirole[f]	1500			79				390	0.05			0.20
SKF-104557[f]	1900			100				1580	0.05			0.06
(+)7-OH-DPAT[d]		3.5	117	3.0[e]						0.86		
(+)7-OH-DPAT[j]	92			2.2					0.02			
(+)7-OH-DPAT[g]	103			2.1					0.02			
(+)PD128,907[d]		7.4	851	2.1[e]						0.28		
(+)PD128,907[j]	339			1.9					0.06			
(+)PD128,907[g]	389			1.8					0.05			

[a] Ratio of the K_i for binding to the hD_3 receptor and the K_i for the hD_2 receptor; > 1.0 equals preference for the hD_3 receptor.
[b] Ratio of the K_i or K_H for binding to the hD_3 receptor and the K_H for the hD_2 receptor; > 1.0 equals preference for the hD_3 receptor.
[c] Perachon et al. (1999).
[d] Newman-Tancredi et al. (1999).
[e] One site model for binding, fit to two-site model not significantly better than to one-site model.
[f] Coldwell et al. (1999).
[g] Sautel et al. (1995a).
[h] Mierau et al. (1995).
[i] Binding utilizing [^{3}H]spiroperidol.
[j] Audinot et al. (1998).

clinical and preclinical literature that supports the hypothesis that the onset of PD is not only correlated with a loss of DA innervation and an elevation in receptor number of the D_2 subclass, but also a decrease in D_3 receptor number. We present evidence that levodopa (L-dopa) treatment tends to normalize D_3 receptor number in early PD. However, with the progression of the pathology, likely including the further loss of mesolimbic DA neurons, we believe that a permanent reduction in D_3 receptor number in critical striatal regions occurs in PD. At that stage, traditional antiparkinsonian drugs are not able to effectively ameliorate many of the predominant symptoms of PD and the patient is judged to be unresponsive to these drugs. Based on new evidence from non-human models of PD, we propose that to overcome the down-regulation in D_3 receptors, an enhancement in current pharmacotherapy for PD would include agonists with a high affinity for this subtype.

With the identification in 1976 that antipsychotics (e.g., Haldol, haloperidol) bind to DA D_2 receptors (Creese et al., 1976; Seeman et al., 1976), it had become widely believed that it is the blockade of one or more members of the D2 family of receptors that alleviates the symptoms of schizophrenia (Joyce & Meador-Woodruff, 1997). It is now apparent that DA antagonists used in the treatment of schizophrenia are not selective for the D_2 receptor, but that they also exhibit high affinity for the D_3 receptor (Table 2). Even in the case of the atypical antipsychotic clozapine, which has 7- to 56-fold higher affinity for the D_2 and D_4 receptors, respectively, as compared with the D_3 receptor, it has predominant actions through neurons expressing the D_3 receptor (Guo et al., 1995, 1998). Thus, occupancy of the D_3 receptor by antipsychotics cannot be excluded as a target site for therapeutic intervention. This follows another hypothetical theme posed by the author that the hyperdopaminergic state of the mesolimbic DA system evokes an up-regulation in D_3 receptors. If D_3 receptor activation can enhance the D_1:D_2 synergism/cooperativity that is observed in c-fos expression (Section 3.2), then one would anticipate an over-reactivity of the D_1 receptor + substance P (Sub P)/dynorphin (DYN)-expressing neurons and an under activity of the D_2 receptor + enkephalin (ENK)-expressing ventral striatal outputs. If this is translated into consequences in the limbic system, it may result in an enhanced thalamic drive to the limbic cortex – as is reported for schizophrenia.

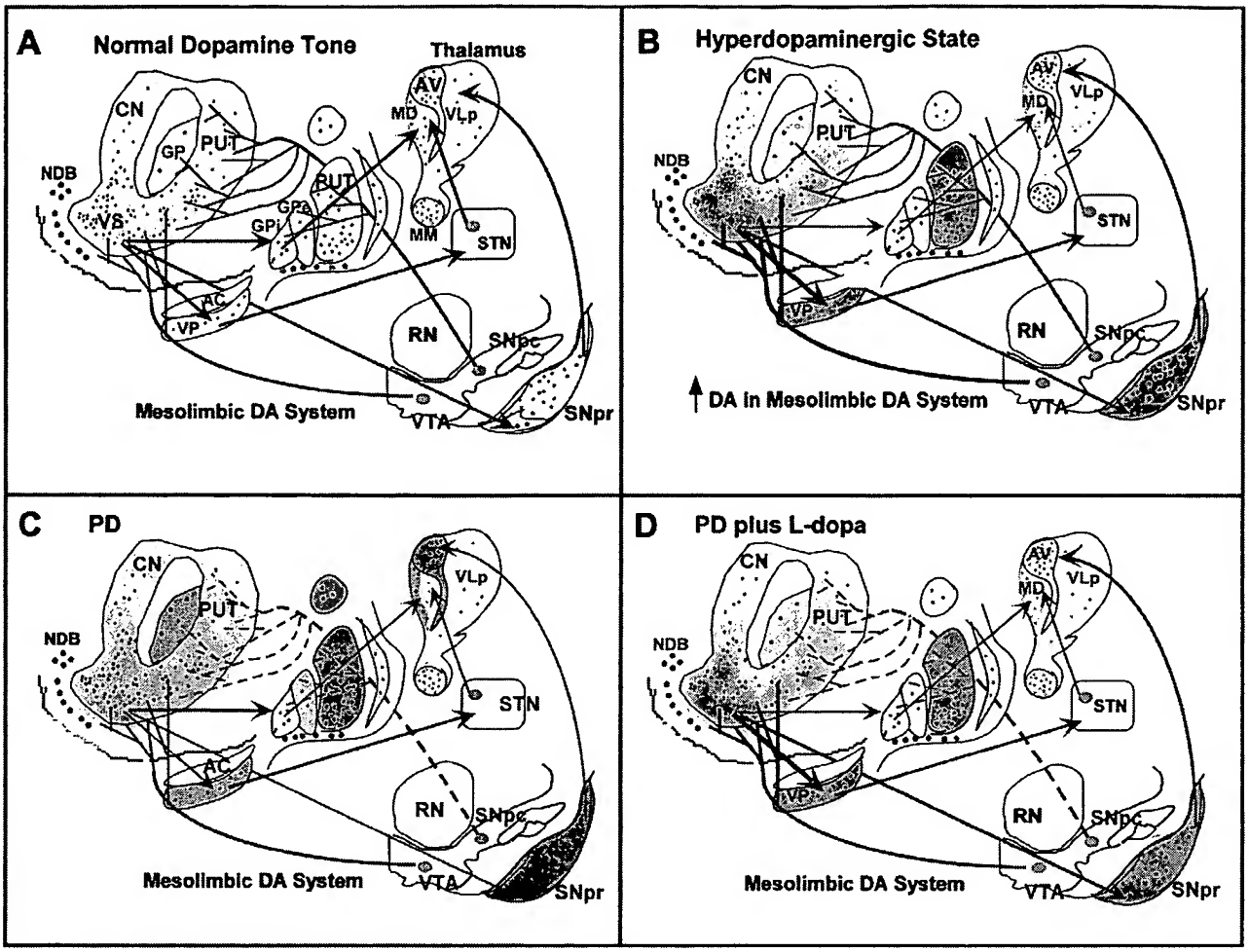

Fig. 1. Schematic diagram of the relative distribution of D_3 receptors and neurons expressing D_3 mRNA in regions of the human forebrain under different conditions of DAergic tone. In each panel, the mesolimbic and nigrostriatal DA systems are shown. Panel A depicts the normal state and relative concentrations of D_3 receptors and D_3 mRNA. The normal levels are depicted in yellow. Panel B depicts the hyperdopaminergic state that occurs with schizophrenia, and is associated with elevated mesolimbic DA, elevated concentrations of D_3 receptors, and neurons expressing D_3 mRNA (red) within the ventral striatum and brain structures that receive its efferents. Panel C delineates the effects of reduced input from the nigrostriatal DAergic system that produces a reduction in D_3 receptor number (blue) in brain areas associated with the ventral striatal circuitry. Panel D depicts the possible hyperdopaminergic state that can occur in PD with the introduction of high concentrations of pulsatile L-dopa and that can produce elevated concentrations of D_3 receptors and neurons expressing D_3 mRNA (red). AC, anterior commissure; AV, anteroventral nucleus of the thalamus; MD, mediodorsal nucleus of the thalamus; MM, mammillary bodies; NDB, nucleus of the diagonal band; PPN, pedunculopontine nucleus; RF, reticular formation; RN, red nucleus; SC, superior colliculus; VL_p, ventral lateral posterior nucleus of the thalamus; VS, ventral striatum.

Thus, D_3 antagonists may prove beneficial in treating this disorder. This review will consider the potential of the D_3 receptor as a therapeutic target for antipsychotics and anti-parkinsonian drugs by reviewing its anatomy in the human brain, proposed role in behavioral processes, regulation, and evidence for a direct role in schizophrenia and PD.

2. Integration of the dopamine D_3 receptor with "limbic" striato-pallidal circuits

It is well accepted that the functions of the striatum are related to its connections with other parts of the brain, particularly with the cortical regions that project to it. Different regions of the cortex provide non-overlapping projections to the striatum segregated into a number of territories (Alexander et al., 1990 Parent & Hazrati, 1995). To a certain degree, these functionally divergent territories have their own downstream targets and differentially express DA receptors. These territories, in turn, have principal DA projections. Two major divisions of the DA systems exist within the basal ganglia, the nigrostriatal and mesolimbic systems (Fig. 2). The caudate putamen (CPu) in the rat and the putamen (PUT) in the primate receive DA projections from the nigrostriatal DA system. The origination of the "nigrostriatal" DA system is restricted to the ventral tier of the substantia nigra pars compacta (SNpc) in primates, and is affected initially and predominantly in PD (Lynd-Balta &

Table 2
Drug affinities (nM) of antagonists to cloned human DA receptors

Drug	hD_1 receptor	hD_2 receptor	hD_3 receptor	hD_4 receptor	hD_5 receptor	D_3:D_2 selectivity
Haloperidol	30	0.3–0.6	3	4–6	40	≈ 0.1
Pimozide		10	11			1
Chlorpromazine		2.3	6			≈ 0.4
Spiperone		0.06	0.25			≈ 0.2
Domperidone		0.5	3.5			≈ 0.1
Clozapine	140	70	500	9	250	≈ 0.1
(−)Sulpride	>40,000	10	20	50	80,000	0.5
Raclopride		1–2	1–3			≈ 1
Risperidone		1–4	5–11			≈ 0.3
Nafadotride	890	3–5	0.3–0.8	269–1780		≈ 10
U 99194		2281	160–223	> 10,000		≈ 10
GR 103,691		24	0.4	81		≈ 60
(+)S 14297		297	13	1380		≈ 23
S33084	500	32	0.25	1900		128
GR218,231	< 1,000	63	1	10,000		63
LY 741,626	700	4	63	300		0.06
SB-271011-A		1047	11.2			93

Data from Audinot et al. (1998), Millan et al. (1995, 2000b), Reavill et al. (2000), Sautel et al. (1995a), Sokoloff et al. (1992), Sunahara et al. (1991), and Van Tol et al. (1991).

Haber, 1994b; Hornykiewicz, 1998). The functions of the terminal regions of the nigrostriatal DA system reflect its sensory-motor cortical inputs, and DA receptor antagonists are thought to produce parkinsonism via blockade of D2-like receptors in this region (Joyce & Meador-Woodruff, 1997). The mesolimbic DA system innervates the nucleus accumbens (NA), the olfactory tubercle, and the Islands of Calleja (ICj) in the rat and the ventral striatum (NA and ventral PUT) in the primate. Based on tracer studies, the mesolimbic DA system of the primate would include the A10 region (ventral tegmental area [VTA], parabrachial pigmented, paranigral nuclei) (Jimenez-Castellanos & Graybiel, 1987; Lynd-Balta & Haber, 1994a, 1994b). The ventral striatum is often termed the limbic striatum because it receives afferents from the orbital and medial prefrontal cortex, the limbic and paralimbic cortices, the thalamus, and the amygdala (Haber & McFarland, 1999), and it integrates these signals under the modulatory influence of the mesolimbic DA system (Lynd-

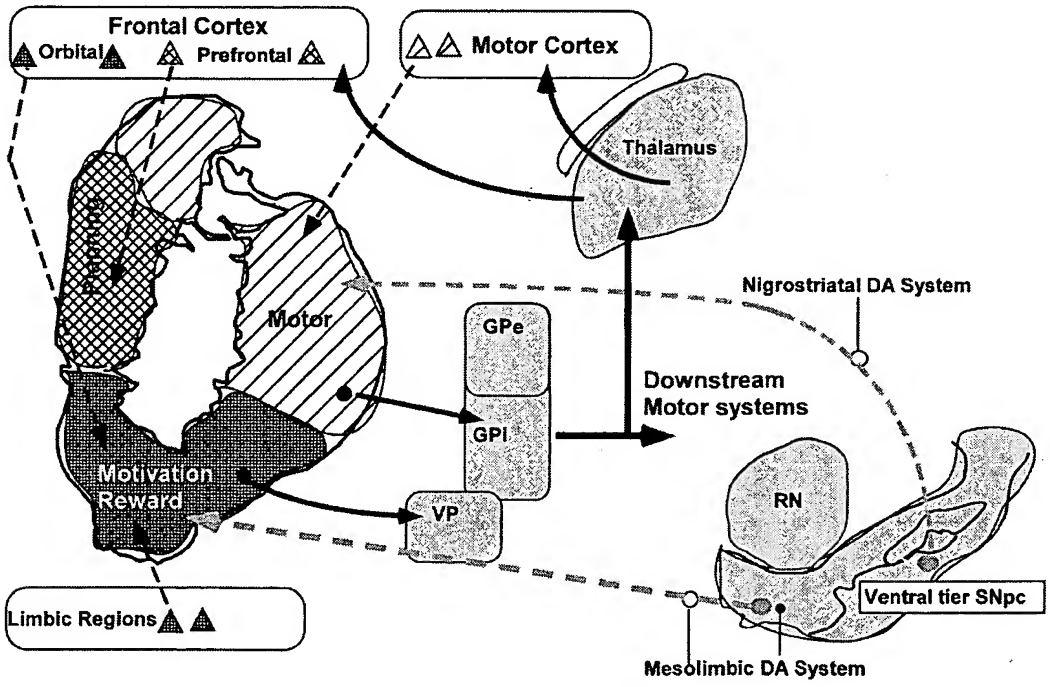

Fig. 2. Schematic diagram indicating the functional organization of the striatum and its cortical and DAergic afferents. Also delineated is the general organization of striatal efferents via the pallidal-thalamic loop and downstream motor pathways. RN, red nucleus.

Balta & Haber, 1994a; Mogenson et al., 1988). The limbic striatum clearly has been demonstrated to be involved in goal-directed behavior, locomotor activity, behavioral sensitization, and changes in affective state. As the efferents of the dorsal and ventral striatum express different types of DA receptors, DA can act to modulate a wide variety of behavioral functions related to motor, cognition, motivation, and affect via different classes of receptors.

2.1. D_1 and D_2 receptors and their striatal efferents

An understanding of how DA agonists and antagonists act in the striatum requires familiarity with its basic organization. As initially postulated by Heimer and Wilson (1975), it has now been convincingly demonstrated that there are parallel efferent circuits originating from the dorsal and ventral striatum with analogous "direct" and "indirect" pathways (Alexander et al., 1990). In the rat, the CPu gives rise to two distinct efferent pathways (Fig. 3). "Direct" pathway neurons project to the entopeduncular nucleus [analogous to the internal segment of the globus pallidus (GPi) in primates] and substantia nigra pars reticulata (SNpr), and express Sub P and preprotachykinin mRNA. "Indirect" pathway neurons project to the globus pallidus [external segment (GPe) in primates] and subthalamic nucleus (STN), and express ENK (preproenkephalin mRNA). In the rat neostriatum, "direct" (striatonigral) and "indirect" (striatopallidal) output neurons preferentially express D_1 and D_2 receptors, respectively, with little overlap (Curran & Watson, 1995; Gerfen et al., 1990; Le Moine & Bloch, 1995). The influences of the direct and indirect pathways are considered to be opposing, and DA tonically modulates these two pathways via inhibition of the indirect pathway through the D_2 receptor and via excitation of the direct pathway through the D_1 receptor (Fig. 3). Similar "direct" and "indirect" pathways have been identified in the limbic striatum. Neurons within the NA project primarily to the ventral pallidum (VP), with the core of the NA (NAC) projecting to the lateral VP, which send projections to the STN and SNpr. The shell of the NA (NAS) projects to the medial VP, which provides efferents to the mediodorsal thalamus (Lu et al., 1998). As cells in the NAS co-express D_1 mRNA and Sub P and the cells in the NAC preferentially express D_2 mRNA and ENK (Curran & Watson, 1995; Lu et al., 1998), they are presumed to modulate direct and indirect ventral striato-pallidal-thalamo pathways, respectively. However, there is a subset of neurons co-expressing ENK and Sub P. Thus, DA probably has separate and convergent influences on limbic striatal efferents.

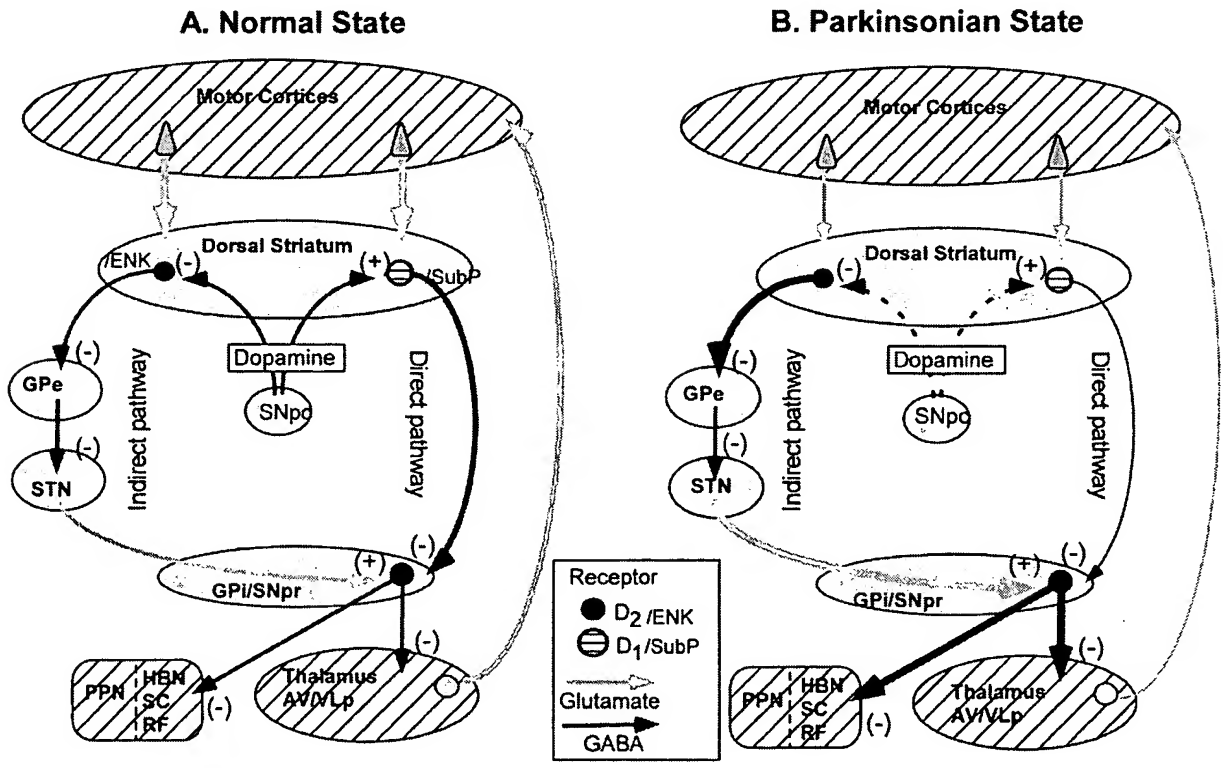

Fig. 3. Schematic diagram of the basal ganglia circuitry that are the components of the striato-pallido-thalamo circuit involved in motor control. The direct and indirect pathways are delineated, as well as the excitatory and inhibitory control by DA on these pathways via D_1 and D_2 receptors that are selectively expressed in neurons of the direct and indirect pathway. The normal state and the consequences of the loss of DA on the direct and indirect pathways that occur in PD are shown. AV, anteroventral nucleus of the thalamus; GABA, γ-aminobutyric acid; HBN, habenular nucleus; PPN, peripeduncular nucleus; RF, reticular formation; SC, superior colliculus; VL_p, ventral lateral posterior nucleus of the thalamus.

2.2. Dopamine D_3 receptor

The DA D_3 receptor is a member of the D2 receptor family, but the evidence strongly favors the complementary distribution of D_2 and D_3 receptors at the regional and cellular level. In the rodent, the D_3 receptor is generally much less abundant in the brain than the D_2 receptor, but the difference is particularly striking in the CPu, where the D_2 receptor is densest. D_3-binding sites and mRNA are densest in the NA, the limbic region of the striatum, whereas in the CPu, D_3-binding sites are low and mRNA is barely detectable (Bouthenet et al., 1991). Lesion studies indicate that D_3 receptors are expressed on cell bodies of the NA and its terminals in the VP and SN (Stanwood et al., 2000a). The NAS, which is the prime source of the "direct" efferents to the medial VP, contains considerably higher concentrations of D_3-binding sites and D_3 mRNA than the core, which is the opposite for D_2-binding sites and D_2 mRNA. Several studies have shown that in the rat NA, the D_3 receptor mRNA is expressed at higher concentrations in neurons expressing the D_1 DA receptor, Sub P, prodynorphin, and neurotensin, with little overlap with D_2 mRNA (Curran & Watson, 1995; Ridray et al., 1998). However, one comprehensive double-in situ hybridization histochemistry study has shown that in the rat NAC, the D_3 receptor is co-expressed with both D_1 and D_2 receptors in Sub P- and ENK-expressing neurons, respectively (Le Moine & Bloch, 1996). In addition, 95% of the neurons of the ICj contain D_3 mRNA, neurotensin, and Sub P, but not D_2 receptors (Landwehrmeyer et al., 1993; Le Moine & Bloch, 1996). Thus, the strongest data indicates that in the rat, the D_3 receptor is preferentially localized in the direct output neurons to the medial VP and to the SN (Stanwood et al., 2000a) expressing the D_1 receptor (Curran & Watson, 1995).

This limited distribution of the D_3 receptor, which has been largely confirmed in other species, suggested that its functions are related to the mesolimbic rather than the nigrostriatal DA system (Sokoloff et al., 1990). In the human brain, the highest expression of the D_3 receptor is in the ventral striatum, but it is also enriched in the association areas of the caudate nucleus (CN) and Put (Gurevich & Joyce, 1999; Meador-Woodruff et al., 1996; Morissette et al., 1998; Murray et al., 1994). Utilizing nonradioactive in situ hybridization histochemistry, we demonstrated that the highest concentration of neurons expressing D_3 mRNA is in the ventral striatum and in the association striatum (Gurevich & Joyce, 1999). This indicates a role for the D_3 receptor in mediating multiple actions of DA (Figs. 1 and 4). It is unknown whether segregation of the D_3 receptor to ventral striatal output neurons that co-express the D_1 receptor mRNA and preprotachykinin mRNA occurs in the nonhuman primate and human brain as it does in rat brain. Our own studies of the D_3 receptor indicate that there is likely to be high overlap of D_3 with Sub P/DYN neurons in the human that also express the D_1 receptor. These neurons likely preferentially project to the medial GPi and to the medial SN (Reiner et al., 1999). Our results also suggest an overlap in the expression of D_3 and D_2 receptors, as D_3 mRNA is expressed in at least 30% of the neurons of the ventral striatum and D_2 mRNA in over 75% of the neurons (Gurevich & Joyce, 1999). Thus, there may be a co-expression of D_3 and D_2 mRNA in a subset of neurons that project to the VP, as well as in Sub P/DYN/D_1 neurons. The D_3 receptor-expressing neurons in the human ventral striatum may give rise not only to the "direct" pathway from the ventral striatum to the medial GPi (which then innervates anteroventral and mediodorsal nuclei of the thalamus), but also to ENK-positive "indirect" projections to the VP (which in turn innervates the STN, the SNpr, and the hypothalamus). However, as the VP also has extensive Sub P innervation, there is likely a D_3/Sub P-containing projection as well. It seems likely that D_1/D_3 receptor co-expression and D_2/D_3 receptor co-expression occurs in different projection (neuropeptide-expressing) neurons of the ventral striatum (Fig. 4), but this needs further testing. We have hypothesized that in human ventral striatum, neurons co-expressing the D_2 and D_3 receptors project to the VP (express ENK) and those co-expressing the D_3 and D_1 receptors (express Sub P and DYN) project to the GPi and to the SN. Presumably, this offers extensive regulation of ventral striatal neurons via different DA receptor subtypes.

One important issue has been whether D_3 receptors are co-localized to DA neurons of the VTA and SN and, therefore, could play a role as a DA autoreceptor. Initial studies in the rat indicated that DA neurons of the rat VTA did co-express mRNA encoding the D_3 receptor (Bouthenet et al., 1991). Other studies indicated very low expression of D_3 mRNA and of D_3 receptor density in DA neurons (Ariano & Sibley, 1994; Diaz et al., 1995; Richtand et al., 1995). Utilizing double localization of D_2 and D_3 riboprobes with immunocytochemical detection of tyrosine hydroxylase (TH) in human SN and VTA, we found few TH-immunoreactive neurons that co-expressed D_3 mRNA, whereas the majority did express D_2 mRNA (Gurevich & Joyce, 1999). Functional studies on slices obtained from human brain have confirmed that release-regulating autoreceptors are of the D_2 subtype in humans (Fedele et al., 1999). Therefore, it is strongly indicated that across all species studied, it is the D_2 receptor and not the D_3 receptor that predominates in regulating tonic DA formation, release, and activity of DAergic neurons. This has been contradicted, however, by a recent study using a polycolonal antibody against the rat D_3 receptor sequence that was utilized to show that the vast majority of TH-positive neurons of the SN and VTA expressed the D_3 receptor (Diaz et al, 2000). Nonetheless, functional studies have been contradictory. For example, intracerebroventricular injection of D_3 antisense to reduce D_3 receptor levels produced increased extracellular levels of DA in the NA, as measured by microdialysis (Ekman et al., 1998), and caused an increase in DA synthesis in the NA (Nissbrandt et al., 1995), suggesting that the D_3 receptor could play an

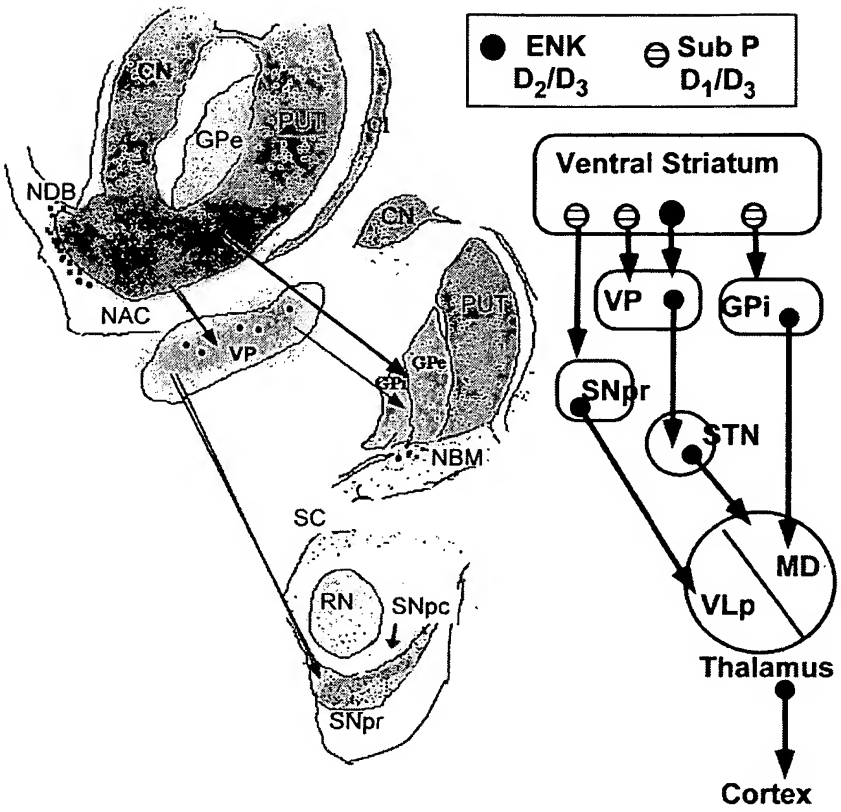

Fig. 4. Integration of the D_3 receptor with the limbic striato-pallidal-thalamo loop. Representation of the relative distribution of D_3 receptors (gray tones) and neurons expressing D_3 mRNA (dark circles and dots) in regions of the human forebrain are shown. Major sources of efferents to the ventral striatum and afferents of the ventral striatal 'limbic' loop are featured. Concentrations of D_3 receptor and D_3 mRNA are highest in the ventral striatum, but are also high within many of the efferents and afferents of the ventral striatum. Cl, claustrum; MD, mediodorsal nucleus of the thalamus; NBM, nucleus basalis of Meynert; NDB, nucleus of the diagonal band; RN, red nucleus; SC, superior colliculus; VLp, ventral lateral posterior nucleus of the thalamus.

autoreceptor role. However, it has also been demonstrated that the D_3-preferring agonist PD128,907 inhibited DA release and that apomorphine inhibited DA formation to a similar extent in D_3 antisense- and missense-injected rats (Ekman et al., 1998; Nissbrandt et al., 1995), consistent with D_2 receptor- and not D_3 receptor-mediated autoreceptor functions. Infusion of D_3 antisense into the SN of rats does not significantly alter the firing rate or pattern of spontaneously active SNpc DA neurons (Tepper et al., 1997). Further studies with the relatively selective D_3 antagonists S33084, GR218,231, and the D_2 antagonist L741,626 support the evidence for the tonic inhibitory role of D_2 autoreceptors and not the evidence for D_3 receptors regulating DA synthesis, release, and activity of DA neurons of the SN and VTA (Millan et al., 2000b). This appears to be consistent with studies in transgenic mice with D_3 receptor deletions (D_3 $-/-$): (1) D2-like agonists have similar effects on DA release in D_3 $-/-$ and wild-type (WT; +/+) mice (L'hirondel et al., 1998), (2) D_3 $-/-$ mice do not exhibit differences in brain levels of DA or its metabolites or differences in levels of the rate-limiting enzyme TH (Jung et al., 1999), and (3) D_2/D_3 agonists are equipotent in inhibiting SN and VTA DA neuronal electrical activity in WT and D_3 $-/-$ mice (Koeltzow et al., 1998). It must be pointed out, however, that a recent study utilizing the D_3 selective antagonist SB-271011-A demonstrated that it reversed the D2/D3/D4 agonist quinelorane-induced reduction of DA efflux in the NA, but not CPu (Reavill et al., 2000). It was also shown to alter the activity of the VTA DA neurons when given subchronically, but not acutely (Ashby et al., 2000). Other investigators have reported that intra-accumbens or systemic low doses of (+)PD128,907 (IC_{25} of 61 nM and 0.05 mg/kg) decreased dialysate DA concentrations in WT mice, which was only apparent at high doses in D_3 $-/-$ mice (IC_{25} of 1327 nM and 0.44 mg/kg), e.g. at doses that would be effective at the D_2 receptor (A. Zapata, J. M. Witkin, & T. S. Shippenberg, unpublished data). Since they failed to find any differences in basal-, potassium-, or cocaine-evoked DA concentrations in D_3 $-/-$ mice as compared with WT mice, this would seem to support a tonic inhibitory role for D_2 autoreceptors and not a role for D_3 receptors in the regulation of DA synthesis and release. Alternatively, the data could be interpreted to support the idea that there is a D_3 autoreceptor, but that it is tonically occupied by DA (Levant, 1995; Stanwood et al., 2000b; Zhang et al., 1999). Because of such high occupancy of the

"presynaptic" D_3 autoreceptor, under basal conditions and psychostimulant stimulation, there is no evident stimulation of this D_3 autoreceptor. Only use of high-affinity/efficacy agonists would allow for the study of the role of the stimulated D_3 autoreceptor in decreased DA release. In this scenario, the D_3 autoreceptor would actually act opposite to the D_2 receptor because it would only be under conditions of low levels of DA that "release" of the D_3 autoreceptor inhibition occurs. Release from inhibition would increase the release of DA to help restore balance. Thus, it could be that the D_2 and D_3 autoreceptors behave as differential controls to the system under high DA release (D_2 inhibiiton) and low DA release (D_3 release of inhibition). This hypothesis should be able to be tested with more selective D_3 receptor drugs to identify the contributions of a D_3 autoreceptor, and/or through a polysynaptic route regulated by D_3 receptive neurons, to functionally regulate DA synthesis, release, and activity of DA neurons of the SN and VTA.

3. Behavioral functions of the dopamine D_3 receptor

The pattern of expression in the brain of the DA D_3 receptor would indicate that behaviors mediated via this receptor should be closely related to the functions of the mesolimbic DA system. Because of this and its close relationship in structure and coupling to intracellular transduction systems of the D_2 receptor, the focus of the analysis of the behavioral functions of the D_3 receptor has paralleled studies of the D_2 receptor. Thus, research has been largely limited to analysis of D_3 receptor mediation of changes in locomotion, although a few other behavioral paradigms have been explored. In addition, the agonists used to test these behavioral effects (e.g., 7-hydroxydipropylaminotetralin [7-OH-DPAT] and PD128,907) show pronounced D_3-D_2 receptor selectivity in vitro (Table 1), but this is far more difficult to establish in vivo. For example, testing of the selectivity of these compounds often does not take into account the multiple affinity states of the D_2 receptor, which may be a better prediction of their in vivo selectivity (Burris et al., 1995; Levant, 1997). When in vitro testing of the discrimination of these agonists is made based on the K_is for the high-affinity state of D_3 and D_2 receptor, the selectivity is reduced as compared with models assuming a single-affinity state for the D_2 receptor (Table 1). Also, while pronounced D_3-D_2 receptor selectivity does exist for these drugs with a variety of functional measures of DA agonist interactions with G-coupled receptors in Chinese hamster ovary cells, the potency of responses in D_3 receptor-transfected cells is often less than that for the D_2 receptor (Coldwell et al., 1999; Newman-Tancredi et al., 1999; Perachon et al., 1999; Sautel et al., 1995a; Vanhauwe et al., 1999). Hence, agonist occupation of D_3 receptors in vivo may be less efficacious than occupation of the D_2 receptor. This observation, coupled with the evidence that there is tonic high-affinity occupancy of D_3 receptors by DA in vivo, at least in the NA (Levant, 1995; Stanwood et al., 2000b; Zhang et al., 1999), makes predictions regarding the behavioral effects of D_3-preferring agonists open to significant revision in the future. Nonetheless, certain concepts have emerged with respect to the functional effects of D_3 receptor stimulation that are important to consider.

The D_3-preferring agonists 7-OH-DPAT and PD128,907 have been reported to decrease locomotor activity at low doses in rats and to increase locomotor activity at higher doses (Daly & Waddington, 1993; Damsma et al., 1993; Depoortere et al., 1996; Gilbert & Cooper, 1995; Khroyan et al., 1995). The behavioral effect of the low dose of these agonists is not associated with a decreased release of DA, suggesting postsynaptic effects of these compounds (Svensson et al., 1994; Thorn et al., 1997), and the same effects occur upon systemic or intra-accumbens injections (Gilbert & Cooper, 1995). Moreover, the D_3-preferring antagonists U99194A and nafadotride increase locomotor activity in rats (Sautel et al., 1995b; Waters et al., 1994). Consistent with the role of the D_3 receptor as being tonically inhibitory for locomotion, the D_3-preferring antagonists AJ76, S 14297, and U99294 do not produce catalepsy and reverse the catalepsy produced by haloperidol (Audinot et al., 1998; Millan et al., 1997). In contrast, behaviors thought to result from sole stimulation of D_2 receptors in the CPu (Dickson et al., 1994), such as high-dose methylphenidate-induced or amphetamine-induced hyperactivity/stereotypes, are not sensitive to suppression by D_3-preferring antagonists (Millan et al., 2000a; Reavill et al., 2000). Locomotor activity mediated by the mesolimbic DA system of the NA appears to be modulated by D_3 receptors, although clearly induced by D_1 receptor stimulation (Swanson et al., 1997). It is hypothesized that the low-dose effects of D_3-preferring agonists occur through the D_3 receptor and higher doses through the D_2 and D_3 receptors, and that the D_3 receptor within the NA exerts inhibitory activity on the behavioral activation to DA agonists (Damsma et al., 1993; Gilbert & Cooper, 1995; Waters et al., 1994). However, it must be pointed out that direct injection of the D_2/D_3 agonist quinpirole in the NAS, a region enriched with D_3 receptors, elicits locomotion at low doses (Swanson et al., 1997). Hence, the specific contribution of D_3 receptor-mediated neuronal systems within subregions of the ventral striatum to DA-initiated behaviors remains to be clarified. In addition, the mechanism of these presumed behavioral effects has been difficult to establish, as behaviors solely attributed to D_3 receptors have not been elucidated in mice with genetic deletions of the D_3 receptor. Thus, for example, D_2/D_3 agonists show similar functional and behavioral responses in D_3 $-/-$ and WT mice (Boulay et al., 1999b; Xu et al., 1999). Recent data, however, suggest that the D_3 receptor may play an important role in mediating the interactions between D_1 and D_2 receptors. In addition, the behavioral action of D_3 receptor-preferring agonists may be altered in animals depleted of DA (Section 6).

3.1. Dopamine D_3 receptors normally dampen D_1:D_2 receptor synergism/cooperativity for dopamine-mediated behaviors

D_3 −/− mice show similar behavioral suppression to D_2-like agonists as WT mice (Boulay et al., 1999b; Xu et al., 1999), but they exhibit a different response to combined administration of D_1- and D_2-like agonists (Xu et al., 1997). Sole stimulation by D_2-like agonists produces inhibition of locomotion (Boulay et al., 1999b), probably through inhibition of DA release via activation of autoreceptors. It is hypothesized that DA D2 agonist-induced locomotor depression is mediated via a DA D_2 autoreceptor-mediated inhibition of DA release onto postsynaptic DA receptors. This reduction in release probably deprives postsynaptic D1- and D2-like receptors of endogenous DA (Jackson et al., 1989). Combined activation of D1 and D2 receptors with D1- and D2-like agonists can act synergistically to increase behavioral activation above either class of drugs given alone in rats (Clark & White, 1987). This appears to be the case in mice also (Starr & Starr, 1989). The D_2 receptor appears necessary for normal locomotion in mice (Boulay et al., 1999a; Jung et al., 1999) and D2-like agonist treatment enhances the locomotor effects of a D1 agonist (Xu et al., 1997). The D_3 receptor appears to modulate this, as in both acute DA-depleted mice (reserpine) and normosensitive mice, the combined administration of D1- (SKF-38393) and D2-like agonists (quinpirole, PD128,907) produced greater activity in D_3 −/− than in WT mice (Xu et al., 1997). In addition, locomotor activity to a range of doses of cocaine resulted in much greater sensitivity in the D_3 −/− than WT mice, as the D_3 −/− mice were responsive to much lower doses of cocaine. Utilizing the conditioned cue preference model for exploring the reinforcing effects of psychostimulants, the same authors showed that D_3 −/− mice exhibited conditioned cue preference to amphetamine at much lower doses than did WT mice. These data suggest that D_3 receptors normally dampen D_1:D_2 receptor synergism/cooperativity.

Further support for this comes from "knockdown" approaches to gene expression of the D_3 receptor in rats. Antisense to the D_3 receptor gene sequence administered intracerebroventricularly to selectively reduce levels of the D_3 receptor in adult rats increased locomotor activity in a novel environment and delayed the habituation-induced reduction in locomotor activity (Ekman et al., 1998). In rats treated acutely with reserpine and α-methyl-p-tyrosine to deplete DA or with reserpine to partially deplete DA, apomorphine administration restored locomotor activity to a greater extent in D_3 receptor antisense-injected rats (Menalled et al., 1999). This can be interpreted to mean that combined D1- and D2-like receptor stimulation produces greater reversal of akinesia in adult rats depleted of DA in the D_3 receptor "knockdown" rats because of an absence of the D_3 receptor that dampens D_1:D_2 receptor cooperativity/synergy. Studies in D_3 −/− mice indicate that there is not a modification of the synergistic effects of the D1 and D2 agonists on NA neurons in D_3 −/− mice as measured by electrophysiology, suggesting that this effect is not occurring at the single cell level (Xu et al., 1997). Interestingly, D_1 receptor-mediated effects on c-*fos* expression appears to be modulated by the D_3 receptor in D_1 receptor -expressing neurons of the NA, but those results are contradictory to behavioral studies (see Section 3.2).

It is possible that D_3 receptor interactions with D_1:D_2 receptor cooperativity/synergy could occur downstream at the level of the VP. This brain region is enriched with D_3 receptors, and at least some of the D_3 receptors are expressed by local neurons in rats (Larson & Ariano, 1995; Diaz et al., 2000) and humans (Gurevich & Joyce, 1999). Electrophysiological data have demonstrated the presence of D_1, D_2, and D_3 receptors in the VP (Napier & Maslowski-Cobuzzi, 1994; C. Napier, unpublished findings). Intracerebral administration of selective D_1 and D_2/D_3 agonists and antagonists into the VP confirm that these DA-receptive neurons can functionally alter behavioral output (Gong et al., 1999; Napier, 1992; Napier & Rehman, 1992). The data also suggest that VP D_1 and D_2 receptors may be located on different neurons (Napier & Maslowski-Cobuzzi, 1994), have different electrophysiological effects (Napier & Maslowski-Cobuzzi, 1994), and are coupled with different, if not opposite, behavioral output (Napier & Rehman, 1992; Gong et al, 1999). D_3 receptors located on D_1-receptive and D_2-receptive neurons in the VP could enhance or suppress their output, resulting in changes in D_1:D_2 receptor cooperativity/synergy.

3.2. Dopamine D_3 receptors normally enhance D_1:D_2 receptor synergism/cooperativity for dopamine-mediated c-*fos* expression

An increase in neuronal activity triggers the transcription of immediate early genes (IEGs), including the proto-oncogene c-*fos*, which, in turn, stimulates the transcription of the activator protein-1 promotor containing late response genes that are responsible for adaptive changes in mature neurons. DA is known to regulate expression of c-*fos* in the striatum via D1 receptor-induced activation and D2 receptor inhibition (Moratalla et al., 1996). However, D2 receptor-mediated effects exhibit both inhibition and activation of c-*fos* (Wirtshafter & Asin, 1994). A form of synergism or cooperativity normally exists between D1- and D2-like receptors (Gerfen et al., 1995; Wirtshafter & Asin, 1994) in c-*fos* expression, as well as a true independent D1 receptor-induced activation and D2 receptor-inhibition (Moratalla et al., 1996). In a careful study, Gerfen and colleagues (1995) showed that induction of IEG zif 268, like that of c-*fos*, was inhibited by the D2-like agonist quinpirole, but showed synergistic activation with a D1 agonist in DA-depleted rats. They then determined that these effects occurred in separate populations of striatal neurons, i.e., ENK-positive (presumptive D_2 receptor posi-

tive; Gerfen et al., 1990; Le Moine et al., 1990) and ENK-negative neurons (presumptive D_1 receptor, DYN/Sub P positive; Le Moine et al., 1990, 1991). Inhibitory effects of a D2-like agonist on zif 268 expression occurs in ENK-positive neurons, and the synergistic activation effects of D1- and D2-like agonists occurs in ENK-negative, presumptive D_1 receptor-expressing neurons. Priming by D1 agonists or mixed D1/D2 agonists leads to activation effects of c-*fos* by D2-like agonists that cannot be produced by priming with D2 agonists (Pollack & Yates, 1999). These data suggest that there are functionally two populations of D2 receptive neurons that modulate D1-mediated effects on c-*fos* expression in striatal neurons.

As discussed in Section 2.2 the D_3 receptor is expressed predominantly in D_1 receptor-positive and DYN/Sub P-containing neurons, and one might expect modified effects of D1 versus D2 receptor-mediated control of c-*fos* levels in D_3 −/− mice. Jung and Schmauss (1999) examined c-*fos* levels in WT and transgenic mice that have D_2, D_3, and D_2/D_3 receptor deletions. The data indicated that there was a blunted c-*fos* response in D_3 −/− mice to the D1 agonist SKF-82958, but the effects of the D_2/D_3 antagonist eticlopride were similar in WT and D_3 −/− mice. These results indicate that although the induction of the c-*fos* response is dependent on D1 receptor stimulation (Moratalla et al., 1996), the maximum c-*fos* response requires a steady-state activity for both D_2 and/or D_3 receptors. D_3 receptor stimulation has been shown to induce c-*fos* expression in primary cultures of rat striatal neurons, albeit at much lower levels than D1 receptor stimulation (Morris et al., 2000). The induction, which occurs in a subpopulation of neurons, may occur in D1 receptive neurons, as it was shown that activation of D1 and D2 receptors, respectively, stimulate and inhibit c-*fos* expression in primary cultures (Simpson & Morris, 1995). Jung and Schmauss' data indicate that the D_3 receptor may play a predominant role in the cooperativity between D1- and D2-like receptors on IEGs, particularly with respect to augmenting D1 receptor induction of c-*fos*. Thus, the inhibitory actions of D2-like agonists may occur through the D_2 receptor on ENK-positive neurons and the synergistic effects with D1 agonists through D_3 receptors on DYN/Sub P neurons. If so, it is difficult to reconcile with the behavioral studies discussed in the previous section that activation of the D_3 receptor dampens D_1:D_2 receptor cooperativity.

Part of the problem in reconciling these different sets of studies is that genetic deletion or antisense reduction of the D_3 receptor produces effects in all classes of neurons that express the D_3 receptor. D_1:D_3 receptor and D_2:D_3 receptor interactions in separate populations of neurons (direct and indirect pathways) are both altered, and these combined effects are difficult to systematically explore. In addition, conditions that best show D2-like receptor augmentation of D_1 receptor induction of c-*fos* in the whole animal, such as depletion of DA and/or chronic DA treatments (Canales & Graybiel, 2000; Gerfen et al., 1995; Pollack & Yates, 1999), also lead to elevation in D_3 receptor number (Section 4). Those conditions also appear to lead to augmentation of D1 receptor-mediated behavioral sensitization that is inhibited by blockade or antisense reduction of the D_3 receptor (Section 5). Thus, D_3 receptor-mediated augmentation of D1 receptor induction of c-*fos* and behavior may be most apparent with depletion of DA and chronic-agonist treatment. It may be that D_1:D_3 receptor and D_2:D_3 receptor interactions in separate populations of neurons (i.e., direct and indirect pathways) change depending on chronic increases and decreases in the levels of DAergic activity. It would be useful to systematically explore D_1:D_3 receptor and D_2:D_3 receptor interactions on c-*fos* and behavior in the whole animal under different conditions of chronic DAergic activity.

4. Regulation of the dopamine D_3 receptor

Key to our understanding of how drugs interact with the D_2 receptor has been our knowledge of the tonic regulation of the D_2 receptor by DA. Less is known about regulation of the D_3 receptor than the D_2 receptor, but current data suggest very differing aspects of control by DA. Experimental parkinsonism induced by intracerebral injection of 6-hydroxydopamine (6-OHDA) in rats (Joyce, 1991a; LaHoste & Marshall, 1991) or systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to monkeys (Gagnon et al., 1995; Graham et al., 1990; Joyce et al., 1986) to produce depletion of striatal DA have consistently demonstrated prolonged elevation in D_2 receptor number in the dorsal lateral CPu of the rat and in the striatum of primates. Such animal models of PD have been utilized to conclude that the elevation in the D_2 receptor number occurs as a consequence of elevated levels of mRNA encoding the D_2 receptor (Angulo et al., 1991; Gerfen et al., 1990; Morissette et al., 1996; Qin et al., 1994), and this occurs in ENK-containing neurons of the indirect pathway (Gerfen et al., 1990). The elevation in D_2 receptor number is restricted to the lateral CPu in rats and the PUT in primates (Gagnon et al., 1995; Graham et al., 1990; Joyce et al., 1986), suggesting intrinsic differences in the response of neurons in that region to the loss of DA innervation. Studies of the regulation of the D_3 receptor have similarly utilized the intracerebral administration of 6-OHDA to rats or of MPTP to monkeys to produce almost complete loss of DA innervation to the striatum. In contrast to that found for the D_2 receptor, D_3 receptor number and mRNA levels are markedly decreased by DA denervation in rats and monkeys (Lévesque et al., 1995; Morissette et al., 1998; Stanwood et al., 2000a; Quik et al., 2000). Importantly, it may not be the loss of DA, per se, that causes the loss of the D_3 receptor. Schwartz and colleagues (Lévesque et al., 1995) demonstrated that striatal D_3 receptor and mRNA loss did not occur with DA receptor blockade (see below), but does occur with blockade of anterograde axonal

transport of proteins in the mesostriatal DA system by intranigral injections of colchicine. Interestingly, a 5-day regimen of reserpine treatment to deplete DA levels did not alter D_3 receptor mRNA levels (Lévesque et al., 1995), but it has been shown that a 14-day treatment lowers D_3 receptor number (Stanwood et al., 2000b). We have also examined D_3 receptor expression in the Zitter rat, which is an autosomal-recessive mutant derived from the Sprague-Dawley rat strain that shows increased free radicals in the SN (Gomi et al., 1994) and progressive age-related loss of the DAergic and serotoninergic innervation to the striatum (Ueda et al., 1998, 2000). We observed significant losses of D_3 receptors, without significant reduction of D_3 mRNA, by 6 months of age in the Zitter rat, with apparently little loss of DA in the NA, but significant reduction of the DA transporter (DAT) located on the fibers of the mesolimbic DA system (Joyce et al., 2000). Hence, it may be that it is not the loss of DA, per se, that controls expression of the D_3 receptor, but other factors transported and released by DA fibers. One suggestion is that of brain-derived neurotrophic factor (BDNF) (Guillin et al., 2001). The transport and release of this factor(s) may also be altered by 6 months of age in the Zitter rats, and this is sufficient for postsynaptic neurons to reduce the expression of D_3 receptors (Fig. 5). This could also occur with long-term reserpine treatment

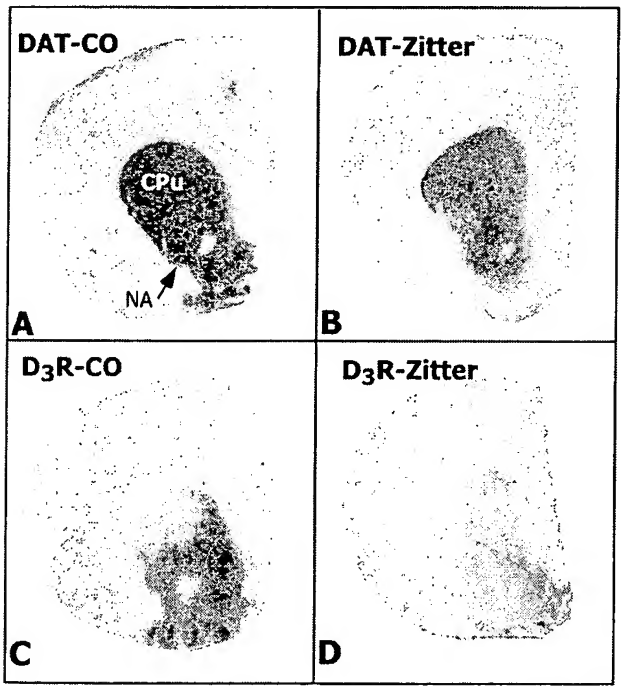

Fig. 5. Photomicrographs of the original autoradiographs for [^{125}I]RTI-55 binding DAT (A, B) and for [^{125}I]trans 7-OH-PIPAT binding D_3 receptors (C, D) in sections from Sprague-Dawley (A, C) and Zitter (B, D) rats at 10 months of age. Note the heterogeneous loss of DAT binding in the CPu and NA of the Zitter rat and the marked reduction of D_3 receptor number. CO, control.

that could lead to disturbances in transport/storage of proteins in DAergic fibers. In summary, this reduction in D_3 receptor number is different from the regulation of the D_2 receptor that occurs in DA-denervated striata in several ways. First, up-regulation of the D_2 receptor occurs and requires >80% depletion of DA and DA innervation to the CPu (Joyce, 1991a). Second, the up-regulation of the D_2 receptor is accompanied by an elevation of D_2 mRNA (Angulo et al., 1991; Gerfen et al., 1990; Lévesque et al., 1995; Morissette et al., 1996; Qin et al., 1994). The reduction in the D_3 receptor is not necessarily accompanied by a similar reduction in D_3 mRNA levels nor does it require as extensive a loss of DA innervation. Moreover, reversal of the D_3 receptor loss in DA-denervated striata can be produced by L-dopa or by repeated D_1 receptor stimulation, but not by D_2 receptor agonists (Bordet et al., 1997; Morissette et al., 1998; Quik et al., 2000). Thus, conditions for regulation of the D_3 receptor appear to be very different from that of the D_2 receptor. In fact, conditions are more similar to that of the D_1 receptor, wherein almost complete DA denervation or intranigral injection of colchicine produces a loss of D_1 receptor number (Joyce, 1991a, 1991b). Given the evidence that there is extensive co-localization of the D_3 receptor in neurons bearing the D_1 receptor, it is possible that there is cross-regulation of the receptors.

This divergent regulation of D_2 and D_3 receptors observed with DA depletion is also the case with chronic receptor blockade for short or prolonged periods of time in rats (Angulo et al., 1991; Florijn et al., 1997; Laruelle et al., 1992; Tarazi et al., 1997). Chronic receptor blockade causes an elevation in the D_2 receptor in the striatum, but the compelling data are that D_3 receptor levels are uninfluenced by short-term administration of DA receptor blockers (Damask et al., 1996; Fishburn et al., 1994; Lévesque et al., 1995). While short-term chronic administration (2-3 weeks) of haloperidol elevates the D_2 receptor in the CPu, it does not appear to elevate it in the NA (Rogue et al., 1991; See et al., 1996), at least at lower doses. However, higher doses (Lévesque et al., 1995) or prolonged chronic treatment with haloperidol (Florijn et al., 1997; Laruelle et al., 1992; Tarazi et al., 1997) does lead to persistent elevation of D_2 receptor number in the CPu and NA. The increase in D_2 receptor number does not always lead to an elevation in D_2 mRNA (Creese et al., 1992; Fox et al., 1994; Matsunaga et al., 1991), although higher doses and more prolonged exposure does elevate D_2 mRNA (D'Souza et al., 1997; Damask et al., 1996; Egan et al., 1994; Lévesque et al., 1995; Rogue et al., 1991). It is likely that a change in post-translational processing of D_2 receptors also contributes to elevated numbers (Pich et al., 1987).

While regulation of D_3 receptors by chronic receptor blockade has not been studied extensively, three studies have examined the consequences of short-term high-dose (Lévesque et al., 1995) or long-term lower-dose haloperidol treatment (Florijn et al., 1997; Tarazi et al., 1997). None of

these studies identified an elevation of D_3 receptors in the NA, olfactory tubercle, ICj, or CPu. Similarly, the majority of studies have not found that short-term administration (7–21 days) of haloperidol elevated D_3 mRNA (Damask et al., 1996; Fishburn et al., 1994; Fox et al., 1994; Lévesque et al., 1995; but see Wang et al., 1996), although one study has suggested that higher doses of haloperidol can elevate D_3 mRNA (D'Souza et al., 1997). We have also completed studies regarding prolonged administration of depot Haldol deconoate (given i.m.) for 9 and 11 months, and with varying withdrawal periods (Joyce, 2001). Again, D_2 receptor binding was elevated in the dorsal striatum, but D_3 receptor binding/mRNA levels were unaltered in the NA or ICj, regardless of the length of time of Haldol treatment or withdrawal period. Chronic treatment with quinpirole or 7-OH-DPAT (D_2-like agonists) also leads to different effects on D_2 and D_3 receptors (Stanwood et al., 2000b). Down-regulation of the D_2 receptor occurred in the NA, but was without effect on D_3 receptor number. It is important to note that these authors did report up-regulation of the D_3 receptor in the VP and SN following the chronic D_2 receptor-like agonist treatment. They also presented data that administration of the irreversible antag-onist *N*-ethoxycarbonyl-2-ethoxyl-1,2-dihydroquinoline was able to occupy D_3 receptors in the VP and SN, but not the NA. This might reflect differences in DA occupancy of D_3 receptors in the VP and SN. As compared with the NA, the D_3 receptor in the VP and SN might be more available for occupancy by DA antagonists. These studies would support the concept that regulation of the D_3 receptor is distinct from that of the D_2 receptor in the NA. Additionally, regulation of the D_3 receptor in the VP and SN might be different from that in the NA.

5. Dopamine D_3 receptor as a target for antipsychotics

Many investigators have proposed that the limbic regions of the striatum, recipient of limbic efferents, play a particularly important role in the gating of disturbed "limbic"-related neuronal processing in schizophrenia (e.g., Mogenson et al., 1988). Since this, in turn, is modulated by the mesolimbic DA system, we (Gurevich et al., 1997; Joyce & Meador-Woodruff, 1997) and others (Schwartz et al., 2000) have proposed that the mesolimbic D_3 receptor mediates this "gate." Our results from postmortem studies supported this hypothesis. First, D_3 receptor mRNA and binding sites are expressed by neurons within the ventral striatum and at several sites in the limbic ventral striatal-pallidal-thalamo circuit (Gurevich & Joyce, 1999; Joyce & Meador-Woodruff, 1997; Meador-Woodruff et al., 1996). Second, our own data demonstrate that there is overproduction of D_3 receptors in the ventral striatum of unmedicated schizophrenic patients (Fig. 6) and those removed from antipsychotics, but down-regulated in those remaining on medication (Gurevich et al., 1997; Joyce et al., 1988; Joyce & Gurevich, 1999). In contrast, D_2 receptors were elevated in both schizophrenic groups, but were somewhat higher in the group withdrawn from medication. Those results are consistent with other postmortem studies that have examined schizophrenics who were treated antemortem with neuroleptics and exhibit an increase in D2-like receptor number (see Joyce & Meador-Woodruff, 1997). The mechanism for the differential response of D_2 and D_3 receptors in this disorder is not known, but certain possibilities can be eliminated. The elevation of D_3 receptors is unlikely due to release from chronic antipsychotic treatment, as we and others (Damask et al., 1996; Fishburn et al., 1994; Florijn et al., 1997; Fox et al., 1994; Lévesque et al., 1995; Tarazi et al., 1997) have not found that D_3 receptor or mRNA levels are elevated with chronic antipsychotic treatment in rats. Secondly, gene polymorphisms in the D_3 receptor and their association with schizophrenia have been identified that may be related to subgroups of patients (Dubertret et al., 1998; Griffon et al., 1996; Jonsson et al., 1999), but many more studies have been negative in this regard (for a review, see Serretti et al., 1999), indicating that gene polymorphisms in the D_3 receptor are not likely to be underlying the changes.

Our hypothesis is that the elevation of the D_3 receptor reflects a hyperdopaminergic state of the mesolimbic DA system. Recent positron emission tomography (PET) imaging studies have confirmed the original hypothesis that the levels of DA are elevated in schizophrenic patients, leading to a hyperdopaminergic tone (Abi-Dargham et al., 1998; Breier et al., 1997; Laruelle, 2000). This appears to reflect alterations in the phasic release of DA, hypothesized to underlie some behavioral disturbances in schizophrenia (Grace, 1993; Laruelle, 2000). Hyperdopaminergic tone induced by pulsatile L-dopa to DA-depleted rats (Bordet et al., 1997) or that in DAT knockout mice with elevated extracellular DA levels (Spielewoy et al., 2000) induce overexpression of D_3 receptors without concomitant elevations in D_2 or D_1 receptors. Moreover, L-dopa-induced sensitization involves elevation of the D_3 receptor in the Sub P/DYN direct striatonigral pathway (Bordet et al., 2000). Abnormal regulation of the mesolimbic D_3 receptor can also reflect modifications in the mesolimbic DA system that occur with partial depletion of the nigrostriatal DA system in early development (Gurevich et al., 1999; Rioux et al., 1997). This also leads to hyperdopaminergic tone of the mesolimbic DA system and to elevated D_3 receptor mRNA as adults. There has been a continuing interest in the possibility that a form of sensitization in the mesolimbic DA system plays an important role in the onset of psychotic symptoms in schizophrenia (Lieberman et al., 1997). It has been proposed that deficits in neural regulation result in a pathological condition of neurochemical sensitization of the mesolimbic DA system analogous to the preclinical model of pharmacologically induced sensitization. It has been well established that pharmacologically induced sensitization (e.g., amphetamine) involves the mesolimbic DA system

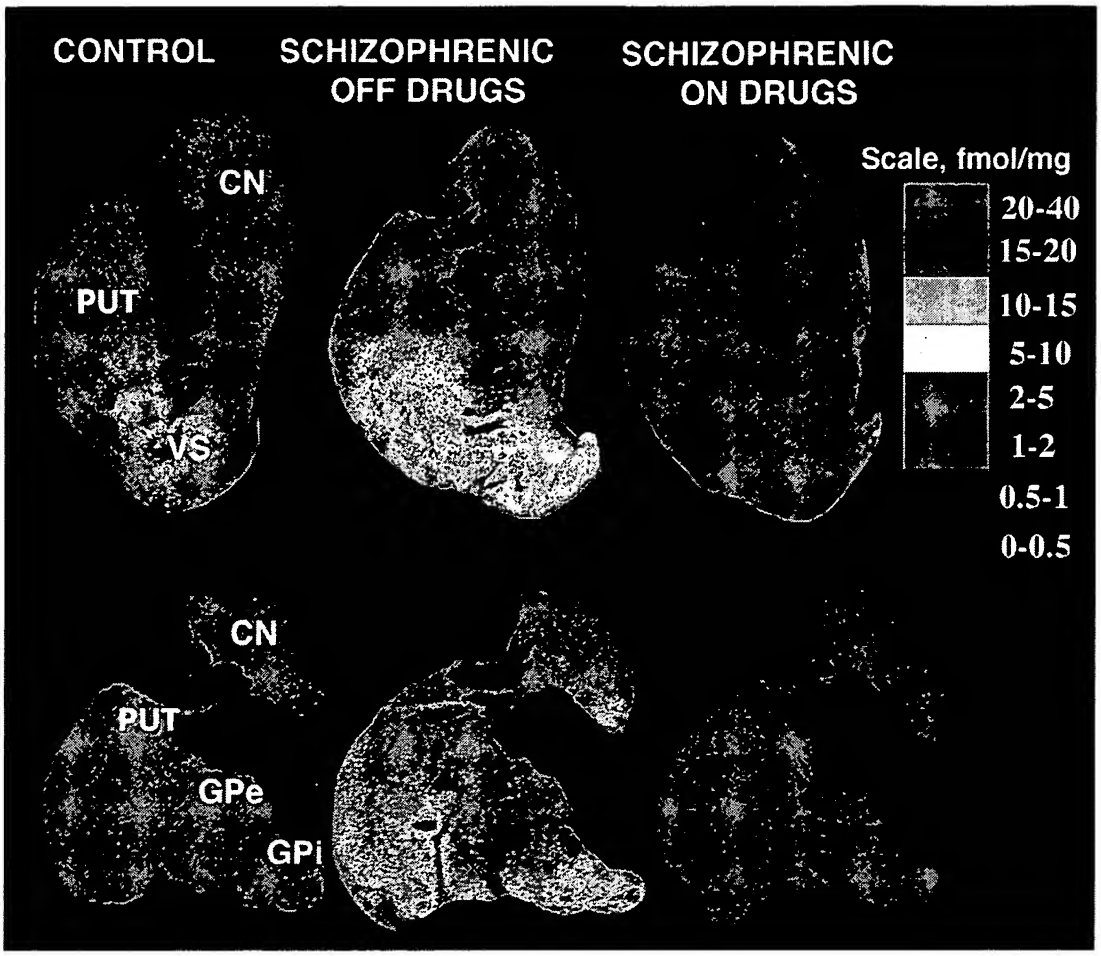

Fig. 6. Pseudocolor photographs of digitized images demonstrating the concentration of D_3 receptors in the striatum of a control patient, schizophrenic patient removed from antipsychotic drug treatment, and a patient remaining on drug treatment at time of death. Tissue is derived postmortem from one rostral level of the striatum (top) and more caudal level (bottom), and processed for D_3 receptor autoradiography. Note the elevated concentrations of D_3 receptors in the ventral striatum and GPi of schizophrenic patients removed from antipsychotic drug treatment. The bar demonstrates the color scale for the concentration of D_3-binding sites. VS, ventral striatum. Data from Gurevich et al. (1997).

and a postsynaptic supersensitivity that is not mediated by an elevation in D_2 receptor number (Kalivas & Stewart, 1991; Robinson & Becker, 1986). It has suggested that sensitization might involve the D_3 receptor (Bordet et al., 1997; Caine et al, 1997). A recent study has shown that the DA D_3 receptor-preferring antagonist nafadotride reduced the ability of amphetamine to produce behavioral sensitization (Richtand et al., 2000). More directly, it has been shown that administration of a D_3 receptor blocker (Bordet et al., 1997) or antisense to reduce D_3 receptor number (Van Kampen & Stoessl, 1999) in L-dopa-primed, behaviorally sensitized rats blocks the behavioral sensitization. Thus, we hypothesize that elevated levels of the D_3 receptor may be caused by the elevated phasic release of DA, and antipsychotics ultimately lead to reduction of the psychotic symptoms via down-regulation of the D_3 receptor (Fig. 1B). We would predict an over-reactivity of the D_1 receptor + Sub P/DYN-expressing neurons and an under-activity of D_2 receptor + ENK-expressing ventral striatal outputs in schizophrenia. If this is translated into consequences in the limbic system, it may result in an enhanced thalamic drive to the limbic cortex. Thus, D_3 antagonists may prove beneficial in treating this disorder. We suggest that antipsychotics are capable of restoring balance to the mesolimbic DA system through multiple sites and mechanisms that ultimately lead to down-regulation of the D_3 receptor. While chronic antipsychotic treatment does not down-regulate NA D_3 receptor number in non-sensitized rats, it is possible that it would do so in sensitized animal with elevated D_3 receptor number.

One of the major caveats with this hypothesis is that none of the currently used antipsychotics are D_3-preferring, and clozapine has higher affinity for D_2 and serotonin (5-HT) receptors than the D_3 receptor. Nonetheless, the clinical efficacy of clozapine may be attributed to its actions on neurons expressing the D_3 receptor (Guo et al., 1995). In a

sophisticated double-localization study of c-*fos* with D_3 receptor mRNA, ENK or DYN in neurons of the NA, ICj, lateral septum, and prefrontal cortex, Guo and colleagues (1998) determined that haloperidol and clozapine induced c-*fos* in separate populations of neurons, even in overlapping regions. For any brain region, the majority of clozapine-induced c-*fos* immuno-like neurons also expressed D_3 receptor mRNA, whereas this was rare for haloperidol. They persuasively argued that both haloperidol and clozapine likely target two distinct subpopulations of c-*fos* immuno-like neurons in the NA, D_3 receptor-positive neurons that express either ENK or DYN for clozapine and D_2 receptor-positive/ENK-positive neurons or D_3 receptor-negative/DYN-positive neurons for haloperidol. It is not evident how clozapine elevates c-*fos* in D_3-positive neurons, as D_3 receptor stimulation has been shown to induce c-*fos* expression in primary cultures of rat striatal neurons (Morris et al., 2000) and D_3 receptor knockout mice exhibit reduced D1 agonist-induced c-*fos* expression (Jung & Schmauss, 1999). Thus, D_3 receptor blockade is unlikely to directly produce the elevation in c-*fos* to clozapine. However, clozapine does preferentially elevate synaptic DA in the NA (Broderick & Piercey, 1998; Moghaddam & Bunney, 1990; Yamada et al., 1995), which could act through D_1 receptors to elevate c-*fos* (Moratalla et al., 1996). This may reflect the importance of the serotonergic properties of clozapine and of other typical antipsychotics such as chlorpromazine in the indirect regulation of D_3 receptors via the mesolimbic DA system. Blockade of 5-HT receptors increase the basal DA levels in the NA via antagonism of the tonic inhibitory control of the mesolimbic DA system by 5-HT (Di Giovanni et al., 1999, 2000; Laruelle, 2000). We determined that schizophrenic patients on antipsychotic treatment have dramatically decreased concentrations of 5-HT receptors in their prefrontal cortex compared with those not taking antipsychotics (Gurevich & Joyce, 1997). We speculate that blockade or even down-regulation of 5-HT receptors by antipsychotics results in tonically elevated mesolimbic DA, which reduces the elevated phasic release of DA (e.g., Grace, 1993) and, in turn, returns D_3 receptor number to normal levels. This suggests that a common mechanism of antipsychotics is to down-regulate D_3 receptors localized to Sub P/DYN neurons and return balance to ventral striatal outputs. This could directly occur through D_3 receptor antagonism or reduced phasic release of DA. DA antagonists used in the treatment of schizophrenia are not selective for the D_2 receptor, but also exhibit high affinity for the D_3 receptor (Table 2) and are capable of direct antagonism of the D_3 receptor (Millan et al., 1995). It would be useful to test if more selective D_3 antagonists, such as S33084 and SB-271011-A, would be effective antipsychotics. If so, we would predict that extrapyramidal side effects associated with nonselective D_2/D_3 receptor antagonists would not be displayed by D_3-selective antagonists, based on the limbic localization of the D_3 receptor.

6. Dopamine D_3 receptor as a target for antiparkinsonian drugs

PD is a neurodegenerative disorder with an insidious onset and a prolonged course over many years. The primary cause of the symptoms of this illness is the death of DA-producing neurons of the SN and the resultant depletion of DA in the striatum (Hornykiewicz, 1998). The therapeutic intervention for PD is based on the assumption that activation of postsynaptically located DA receptors will provide some return of balance to the system (Fig. 3). The preferred mode of treatment of the symptoms of this illness is with L-dopa, which is taken up by surviving DA neurons and converted to DA, which, in turn, can be stored and released. As discussed in Section 2.1, the neurotransmitter DA is thought to tonically modulate the two major striatal outflow pathways via inhibition of the indirect pathway and excitation of the direct pathway. The influence of the direct and indirect pathways are considered to be opposing, and are thought to function to focus movements, to increase or decrease the probability of movement, and to scale movement (Levy et al., 1997). Loss of DA leads to changes in expression levels for D_1 and D_2 receptors in the striatum and changes in DA-mediated activity of the direct and indirect pathways (Fig. 3). However, for many patients, significant loss of effectiveness in L-dopa treatment and inability to reverse dementia and depression occurs after five or more years of treatment (Fabbrini et al., 1988; Mouradian et al., 1988). In fact, clinical studies show that dementia is highly correlated with greater depressive symptomatology, greater motor deficits, and poorer response to antiparkinsonian medication, suggesting a common basis of deterioration (Aarsland et al., 1996; Ebmeier et al., 1990; Hobson & Meara, 1999; Jellinger & Paulus, 1992; Mayeux et al., 1988; Tandberg et al., 1996, 1997). DA receptor agonists are drugs that can bypass the degenerating neurons, and directly stimulate the intact, although denervated, postsynaptic receptors in the striatum (Fig. 3). This effect would be expected to provide an advantage for DA agonists over L-dopa, and thus, increasing interest in these compounds has been at the center of PD clinical therapeutic research. DA agonists can be used to target specific receptor subtypes so that they possibly may have more specific therapeutic effects and perhaps target certain clinical symptoms.

6.1. D_3 receptors are functionally relevant in Parkinson's disease

Within the basal ganglia, D_2 and D_3 receptors, and only extremely low levels of the D_4 receptor, are expressed (Gurevich & Joyce, 1999; Khan et al., 1998; Meador-Woodruff et al., 1996), indicating that stimulation of D_2 and D_3 receptors prominently contributes to the antiparkinsonian effects of most of the compounds used. DAergic receptor stimulation that results in the relief of the parkinsonian motor signs is most consistently observed with drugs

that interact with D2-like receptors (Loschmann et al., 1992; Nomoto et al., 1985; Vermeulen et al., 1999). In addition, up-regulation of the D_2 receptor by DA deafferentation and down-regulation by DAergic stimulation is consistent with a prominent role of the D_2 receptor in this regard (Alexander et al., 1991; Elsworth et al., 1998; Goulet et al., 1997; Reches et al., 1982, 1984). Results with D1 agonist stimulation in experimental non-human primate models of parkinsonism have been more difficult to interpret, as they produce dyskinesias and their antiparkinsonian relief develops rapid tolerance (Vermeulen et al., 1999). Recent introduction of new D_1 agonists may challenge this assumption, but these agonists are not selective for the D_1 receptor, having only 14-fold selectivity versus D_2 receptors (Shiosaki et al., 1996). Therefore, it is likely that their antiparkinsonian activity occurs through stimulation of D2-like or D_2 and D1 receptors. Within the D2 receptor family, it has been assumed that it was D_2 receptor stimulation that was necessary for antiparkinsonian activity, but DA agonists used in the treatment of PD have, in many cases, as high or higher affinity for the D_3 receptor (Table 1). Thus, it remains tenable that the mesolimbic D_3 receptor could play a role in antiparkinsonian relief. While the motor striatum is involved in sensorimotor function and integration of motor movements, the limbic striatum is also involved in aspects of movement, such as goal-directed behaviors and locomotor activity. It is also involved in aspects of behavioral sensitization and changes in affective state. Lesions to the nigrostriatal pathway and its cells of origin in the ventral SN in PD are associated with rigidity and hypokinesia (Jellinger, 1999; Rinne, 1991; Vingerhoets et al., 1997). Dementia and bradykinesia in PD (Mayeux et al., 1992) are correlated with significant cell loss in the medial nuclei of the SN (Jellinger, 1991; Jellinger & Paulus, 1992; Narabayashi, 1995) or origin of the mesolimbic DA system (Figs. 2 and 7). Thus, the mesolimbic DA system might also be involved in "motor" aspects of PD. As we have proposed that it is the D_3 receptor that remains an important target of the mesolimbic DA system, it is possible that D_3 agonists could modulate the effects of mesolimbic DA activity.

Behavioral studies in normosensitive rats would suggest that D_3 agonists would not be effective in PD, as they may inhibit DA agonist-induced behavioral activation and, possibly, DA release (see Section 3). However, in fact, locomotor stimulatory activity is observed in 6-OHDA-lesioned rats at the same doses of D_3-preferring agonists that are inhibitory in normosensitive rats (Van den Buuse, 1993). The D_3-preferring agonist pramipexole (PPX) can reverse muscle rigidity produced by the combination of reserpine and α-methyl-p-tyrosine to deplete DA or haloperidol to block DA receptors (Lorenc-Koci & Wolfarth, 1999). The D_3-preferring agonist 7-OH-DPAT also reverses catalepsy evoked by reserpine or DA receptor blockers in rats (Maj et al., 1999). The

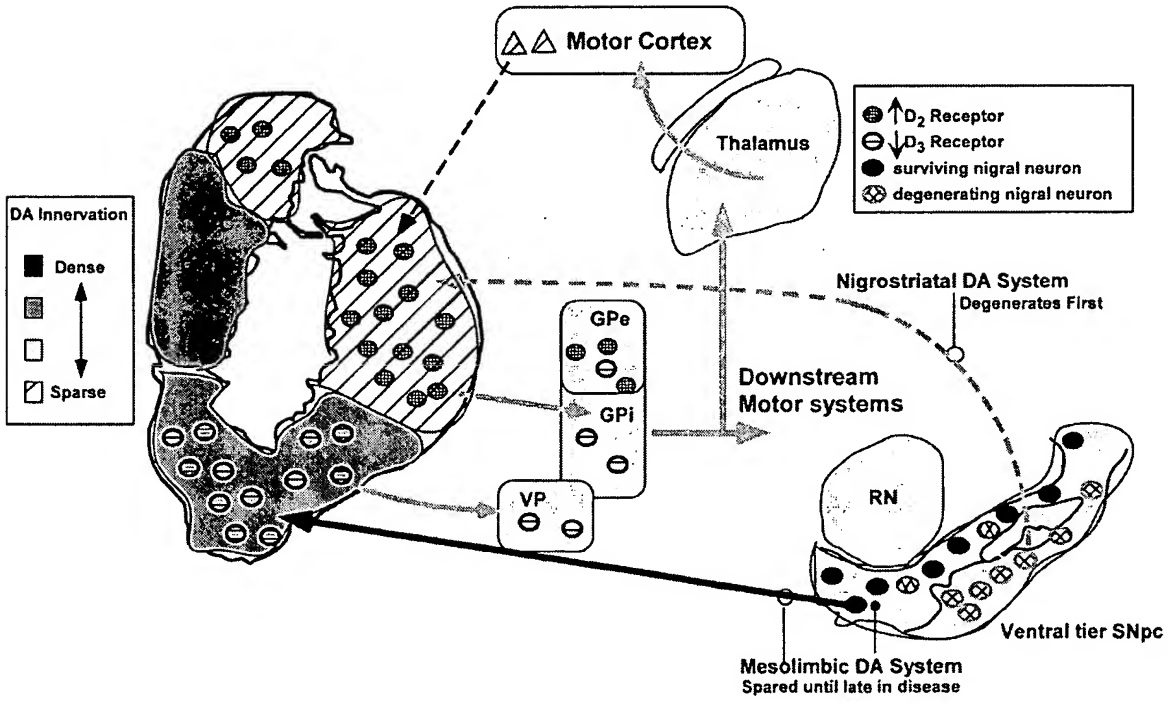

Fig. 7. Pattern of loss of DAergic innervation to the striatum in PD. Schematic diagram of the basal ganglia indicating the pattern of degeneration of DAergic afferents in the striatum and cell body loss in the SN and the VTA is depicted. The nigrostriatal pathway degenerates initially in PD and it is most impacted, leading to a greater loss of DA innervation to the 'motor' subdivision of the striatum. Also depicted are neurons that express the D_2 receptor or the D_3 receptor in different regions of the striatum and different structurss and the impact of the loss of DA fibers. RN, red nucleus.

authors also demonstrated that the D_3-preferring antagonist nafadotride inhibited (1) 7-OH-DPAT-induced hyperactivity, but not the stereotypies induced by amphetamine or quinpirole, and (2) the reversal of reserpine-induced hypoactivity by L-dopa or 7-OH-DPAT. This suggests that animals depleted of DA might respond to D_3-preferring agonists differently from normosensitive animals. This has been further explored in the MPTP model of Parkinsonism in monkeys. In monkeys treated with MPTP to produce severe akinesia, the D_3-preferring agonist PD128,907 was found to be equally potent as apomorphine in reversing the hypoactivity, and this effect was blocked by the D_3 antagonist U-99194A (Blanchet et al., 1997). Similarly, L-dopa reversal of MPTP-induced hypoactivity in monkeys is dose-dependently inhibited by the D_3 antagonist nafadotride (Hadjtahar et al., 1999). In addition, there has been the introduction of the use of D_3-preferring agonists for effective treatment of PD (Hobson et al., 1999; Molho et al., 1995; Shannon et al., 1997). Therefore, it would be of value to explore regulation of this receptor in clinical subgroups of PD.

6.2. Changes in expression of D_3 receptors differ from D_2 receptors in Parkinson's disease

As overviewed in Section 4, D_2 receptors are elevated in animal models of PD. This has been frequently confirmed with in vivo imaging by PET and single-photon emission computed tomography (SPECT) in PD itself, at least in the initial stages (Rinne et al., 1990; Sawle et al., 1993). However, a reduction in D_2-like receptor binding of radioligands has been reported to occur with increased disease duration (Antonini et al., 1995, 1997; Hagglund et al., 1987) and/or complicated response to L-dopa in PD (Brooks et al., 1992; Guttman et al., 1986; Pizzolato et al., 1995). This has led to the speculation that the loss of D_2 receptors contributes to a deteriorated response to L-dopa in PD (Pizzolato et al., 1995). We (Joyce, 1993; Ryoo et al., 1998) and others (Bokobza et al., 1984; Piggott et al., 1999; Seeman et al., 1987) have observed increases of D2-like receptor binding in the range of 25–35% in postmortem studies of PD, regardless of the duration of illness. As overviewed in Section 4, studies in the rat and monkey indicate that D_3 receptors may be regulated by loss of DA afferents differently from the D_2 receptor and that DA fiber loss produces a reduction in D_3 receptor number (Lévesque et al., 1995; Morissette et al., 1998; Quik et al., 2000). As the radioligands utilized in PET and SPECT imaging do not discriminate between D_2 and D_3 receptors, it is not clear if it is a loss of D_2 *or* D_3 receptors that might be correlated with a loss of response to L-dopa in PD. However, direct studies in PD have been mixed with a small reduction of D_3 receptor number reported in one study (Piggott et al., 1999) and no reduction found in another study (Hurley et al., 1996). As these studies did not identify the clinical status of the PD patients, it is possible that changes in D_3 receptor number are associated with clinically defined subgroups.

To examine this further, we explored changes in D_2 and D_3 receptors while simultaneously measuring the extent of DA fiber loss in the striatum of autopsied cases with relatively short or long histories of PD, and found that there was a significant loss of D_3 receptors in PD cases with histories of the illness >10 years (Ryoo et al., 1998). DAergic innervation did not differ between the cases, being equally reduced in those with short (5 years) and long histories. D_3 receptors were reduced by up to 45% in the NA, the ventral PUT, and the GPi. In contrast to the D_3 receptor, the D_2 receptor was elevated in PD by 15% in the dorsal PUT and by 25% in the GPe (Fig. 8). This was consistent with our hypothesis that changes in D_3 receptor number are associated with a clinically defined loss of response to L-dopa, as the decline in response to antiparkinsonian medication is more common in later stages of PD. This could also be associated with the presence of dementia. To provide preliminary information on the relationship between clinical subgroups of PD and changes in D_3 receptor number, we processed tissue from additional PD cases that met clinical and neuropathological criteria for PD

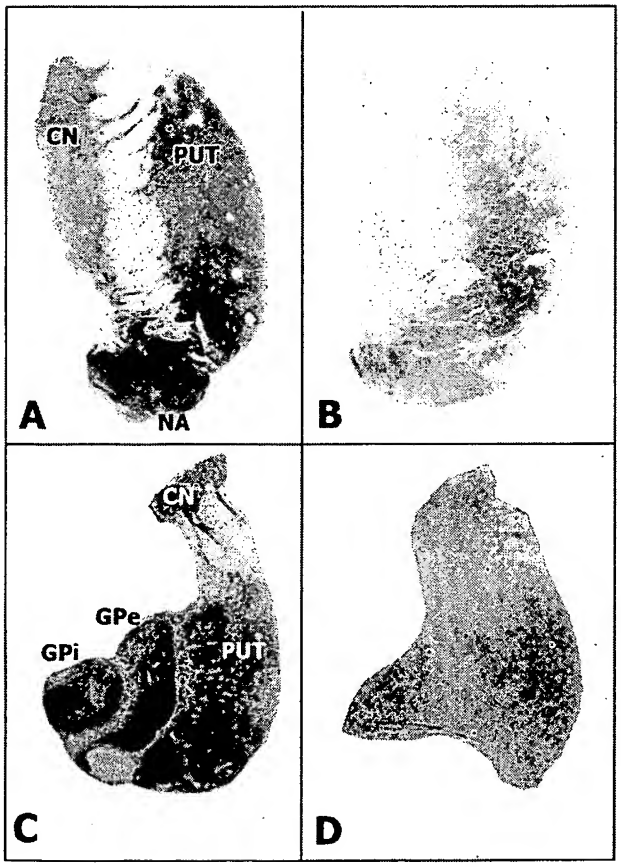

Fig. 8. Photomicrographs of digitized images demonstrating the concentration of D_3 receptors in the rostral striatum of a control (A) or a PD case (B), and in the caudal striatum of a control case (C) and a PD case (D). Note the reduced concentrations of D_3 receptors in the PD patient in the ventral striatum and in the CPu, GPe, and GPi. Data from Ryoo et al. (1998).

and age-matched controls. Clinical information regarding presence or absence of dementia and whether the PD cases had become resistant to the beneficial response of L-dopa was acquired (nonresponder versus continued responder). Measurement of D_3 receptor number showed only a small decline for the PD group as a whole, but significant differences emerged (even with the small number) for the subgroups. PD with an additional diagnosis of dementia (with or without Alzheimer's pathology) *or* categorization as a nonresponder to antiparkinsonian medication was correlated with a lower D_3 receptor number (-48%). In contrast, PD cases that were still responsive to antiparkinsonian medication and nondemented exhibited elevated levels ($+25\%$) of D_3 receptor binding (Joyce et al., 2001).

6.3. Beneficial response to D_3 receptor-preferring agonists in Parkinson's disease

It has been proposed that rigidity, tremor and secondary akinesia (that related to rigidity) start first with degeneration of the ventral tier of the SNpc, followed by cell death in the dorsal tier and medial nuclei (source of the mesolimbic DA system), which, in turn, may produce primary akinesia (bradykinesia and psychomotor retardation), dementia, and depression. This pathology in the mesolimbic DA system might be one of the key factors responsible for the progressive worsening of the disease and the increase in nonresponders with disease duration (Jellinger, 1991; Narabayashi, 1995). Studies in experimental models of PD have found that mesolimbic DA loss is related to deficits in initiation of movement and specific aspects of goal-directed behavior (Carey, 1983; Meeker et al., 1998; Salamone et al., 1997; Wolterink et al., 1990), as well as some aspects of cognitive function (McCullough et al., 1993; Ploeger et al., 1994; Salamone, 1992). While the focus has been on D_2 receptor effects of antiparkinsonian agents, D_3-preferring agonists can be effective in ameliorating motor symptoms in PD patients (Hobson et al., 1999; Molho et al., 1995; Shannon et al., 1997) and MPTP-treated monkeys (Blanchet et al., 1997). The beneficial effects of the D_3-preferring agonist PD128,907 in monkeys treated with MPTP were blocked by the D_3-preferring antagonist U-99194A (Blanchet et al., 1997). Similarly, L-dopa reversal of MPTP-induced hypoactivity in monkeys is correlated with the induction of D_3 receptors (Police et al., 1999; Quik et al., 2000) and is dose-dependently inhibited by the D_3-preferring antagonist nafadotride (Hadjtahar et al., 1999). Hence, regulation of the D_3 receptor may be important in understanding the response of PD cases to antiparkinsonian medication.

We hypothesize that initial expression of parkinsonian signs in PD would be correlated with loss of DA innervation, elevated D_2 receptor number, and decreased D_3 receptor number. L-dopa treatment would normalize/elevate the D_3 receptor number in early PD (Fig. 1C and D). The reduction in D_3 receptors caused by DA depletion in experimental models of PD can be reversed in the NA and dorsal CPu of rats and monkeys by chronic treatment with L-dopa or D_1 agonists, but not with D_2-agonist treatment (Bordet et al., 1997; Morissette et al., 1998; Quik et al., 2000). However, with further neuropathology, likely including the further loss of mesolimbic DA neurons, we believe a permanent reduction in the D_3 receptor number in critical striatal regions occurs in PD (Fig. 7). At that stage of PD, antiparkinsonian drugs are not able to effectively ameliorate many of the predominant symptoms of PD, and the patient is judged to be unresponsive to antiparkinsonian drugs. In our study, loss of D_3 receptors was associated with significant deterioration in response to antiparkinsonian medication and commonly with dementia (typically with Alzheimer's disease pathology present). Piggott and colleagues (1999) and our own unpublished results have determined that the D_3 receptor number is not reduced in the ventral striatum of Alzheimer's disease, so the reduction of D_3 receptors in the PD with dementia group is unlikely to be due to Alzheimer's disease pathology per se. The PD cases reported as poor responders to PD medication all had originally responded well to L-dopa (Sinemet©), were not identified at neuropathology as having progressive supranuclear palsy or other Parkinson-like diagnosis with known poor response to antiparkinsonian medication, and typically also did not respond to direct DA agonists. The high overlap of the cases that had become poor responders to antiparkinsonian medication or exhibited symptoms of dementia suggests a common pathological basis, such as damage to the mesolimbic DA system (Jellinger, 1991), which also leads to a loss of postsynaptically located striatal D_3 receptors. Thus, we believe that reduced D_3 receptor number is correlated with certain subgroups of PD and may also be related to a further diminishment in the mesolimbic DA system. In addition, D_3 receptor stimulation may prove to be beneficial in relieving specific symptoms in PD. A recent double-blind, randomized controlled trial of the comparison of L-dopa with PPX in early PD indicated that there was significantly less development of wearing off or of on-off motor fluctuations in the PPX-treated group at the end of 2 years (Parkinson Study Group, 2000). Additionally, the D_3-preferring agonist PPX appears to have antidepressant effects in animal models (Maj et al., 1997) and in major depression (Corrigan et al., 2000), which may play a part in its effective antiparkinsonian effects (Piercey, 1998; see also Lammers et al., 2000). It should be possible to explore the contribution of a permanent reduction in D_3 receptor number to the inability of antiparkinsonian drugs to relieve parkinsonian signs in appropriate animal models.

Other investigators have suggested that the D_3 receptor also plays a role in the side effects of L-dopa medication in PD. Because of similarities in the response of the D_1 (Joyce, 1991b) and D_3 receptors (Lévesque et al., 1995) to lesion by 6-OHDA and their extensive co-localization in a subset of striatal neurons expressing Sub P (Le Moine & Bloch, 1996; Schwartz et al., 1998), it seems possible that these receptors

are co-regulated. Consistent with this hypothesis, it has been shown that the D_3 receptor number is reduced by DA depletion and can be induced to increase in the NA and dorsal CPu (typically exhibiting low expression) by chronic treatment with L-dopa or a D_1 agonist in DA-depleted rats (Bordet et al., 1997). As the effect of L-dopa can also be prevented by blockade of the D_1 receptor, this elevation might be due to D_1 receptor stimulation and might occur in neurons co-expressing D_1 and D_3 receptors. However, there was not an elevation in D_1 receptor mRNA in Sub P neurons, only an elevation of D_3 mRNA. The elevation occurs in Sub P/DYN neurons, and is associated with elevated levels of prodynorphine/preprotachykinin mRNA (Bordet et al., 2000). The elevation in D_3 receptors corresponded to behavioral changes in these animals. Chronic L-dopa treatment in 6-OHDA-lesioned rats led to behavioral sensitization that could be blocked by the preferential D_3 receptor antagonist nafadotride (Bordet et al., 1997). This has led to the hypothesis that the D_3 receptor plays a direct role in the L-dopa-induced behavioral sensitization, a model for L-dopa-induced dyskinesias (Bordet et al., 1997; Schwartz et al., 1998). Similar conclusions have been reached by Morissette and colleagues (1998) in the MPTP-treated monkey, which exhibits substantial declines in D_3 receptor number that can be reversed with D_1 agonist treatment, but not D_2 agonist treatment. As the D_1 agonist treatment resulted in effective relief of the parkinsonian signs, but also induced dyskinesias, it suggested a role of D_3:D_1 interactions in D_1 agonist-induced dyskinesias. This might suggest that D_3 receptor elevation plays an important role in behavioral sensitization under conditions of excessive DAergic stimulation. In fact, administration of D_3 receptor antisense to L-dopa-primed, behaviorally sensitized rats blocks the behavioral sensitization and reduces the D_3 receptor number (Van Kampen & Stoessl, 1999). This suggests that D_3 receptor stimulation might underlie the development of L-dopa-induced dyskinesias. However, a recent double-blind clinical study of the incidence of dyskinesias in patients who were treated with ropinirole (Requip©) alone or in combination with L-dopa actually demonstrated a reduced incidence of dyskinesias (Rascol et al., 2000). A recent double-blind, randomized controlled trial of the comparison of L-dopa with PPX in early PD also indicated that there was significantly less development of dyskinesias in the PPX-treated group at the end of 2 years (Parkinson Study Group, 2000). Hence, it may be that DA agonist stimulation of the D_3 receptor will not produce behavioral sensitization and/or dyskinesias in the absence of L-dopa-induced behavioral sensitization and/or dyskinesias. Future studies need to explore whether direct modulation of the D_3 receptor might be important in relieving the deteriorating response in PD and in hyperactive conditions such as L-dopa-induced dyskinesias.

It has been claimed that the D_3-preferring agonists PPX and ropinirole may be more likely than L-dopa to produce brief periods of daytime sleep in PD (Frucht et al., 1999; Hauser et al., 2000), and may not be well tolerated by some PD patients. However, considerably more research needs to be conducted in this regard, as it may not be more frequent than with other antiparkinsonian drugs (Olanow et al., 2000). It contrast to these possible controversive reports on the use of these drugs, it has now been established that the D_3-preferring agonists PPX and ropinirole are effective in relieving the symptoms associated with restless leg syndrome, and are superior to that of L-dopa (Montplaisir et al., 1999; Saletu, B. et al., 2000; Saletu, M. et al., 2000). Thus, there may be other clinical conditions for which therapeutic intervention with D_3 ligands are beneficial.

6.4. Neuroprotective actions of D_3 receptor-preferring agonists

An interesting development in the use of DA agonists for the treatment of PD is that some of them may prove to be neuroprotective. Interestingly, antiparkinsonian agents that are direct DA agonists, such as apomorphine (Grunblatt et al., 1999a), bromocriptine (Muralikrishnan & Mohanakumar, 1998), talipexole (Kitamura et al., 1997), and PPX (Kitamura et al., 1997), have been shown to be neuroprotective against MPTP in mice. MPTP administration to mice is considered a good model of studying neuroprotection because MPTP is known to produce parkinsonism in humans and in subhuman species through selective loss of DAergic neurons of the SN (Burns et al., 1983; Langston et al., 1983), there are a number of related compounds to MPTP that produce nigral cell loss in primates (McNaught et al., 1996), MPTP produces biochemical changes associated with oxidative stress similar to that in PD (Cassarino & Bennett, 1999; Cohen & Werner, 1994), and MPTP produces ongoing cell death in humans for decades after the initial insult (Langston et al., 1999). Hence, drugs that reduce the neurotoxicity of compounds, such as MPTP or its active metabolite *N*-methyl-4-phenylpyridinium (MPP+), may prove to be neuroprotective in PD.

Neuroprotection against MPTP was initially demonstrated by showing that it prevented the reduction of the MPTP-induced striatal DA depletion and striatal TH immunoreactivity in mice (Kitamura et al., 1997; Lange et al., 1994; Muralikrishnan & Mohanakumar, 1998). The D_3 receptor-preferring agonist PPX appears to be the most potent, showing neuroprotection at a dose of 1 mg/kg (Kitamura et al., 1997; Zou et al., 2000) as compared with apomorphine (10 mg/kg; Grunblatt et al., 1999a) and bromocriptine (30–100 mg/kg; Muralikrishnan & Mohanakumar, 1998; Lange et al., 1994). We recently have extended those findings by examining the effects of PPX on MPTP-induced neurotoxicity in very old mice. Previous studies of neuroprotection afforded by DA agonists in MPTP-treated mice have utilized relatively young animals. This may not be the most appropriate model because it is aged humans that are predominantly affected by PD. We examined whether truly aged mice are also provided protection by the D_3-preferring

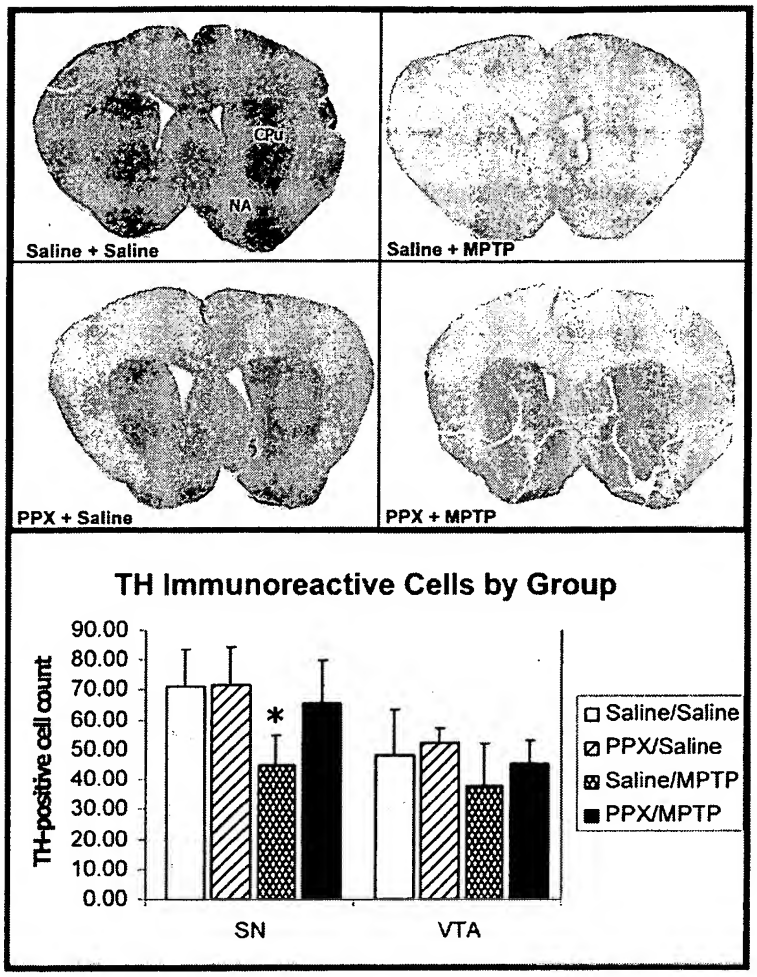

Fig. 9. Photomicrographs (top) and bar graph (bottom) demonstrating the impact of PPX pretreatment on markers of neurotoxicity induced by MPTP in aged mice. A single daily dose of PPX or saline at a dose of 3 mg/kg body weight was given for 3 days prior to MPTP (or saline) treatment and for 3 days after treatment. Four injections of MPTP for a total dose of 80 mg/kg were administered per animal, and tissue was collected at the end of the 2-week recovery period. Striatal sections of aged mice pretreated with PPX or saline and then injected with MPTP or saline were processed for DAT immunoreactivity (top). Note the marked reduction of DAT immunoreactivity in the CPu and NA of saline-pretreated mice administered MPTP (saline + MPTP) in comparison with mice treated with saline + saline, PPX pretreatment followed by MPTP (PPX + MPTP), or PPX pretreatment followed by saline (PPX + saline). Mean ± S.D. for the cell counts in the SN and VTA of the same groups of mice for sections processed for TH immunoreactivity are shown (bottom). There was a significant loss of TH immunoreactive cells in the SN ($P < 0.05$) of the saline + MPTP group, but not in the other groups.

agonist PPX by testing its effects against MPTP-induced DA neuron cell death in 20- to 24-month-old B6C3F1 mice (J. N. Joyce, S. Presgraves, and H. L. Ryoo, unpublished data). Pretreatment with PPX significantly, but not completely, reduced the impact of MPTP on DAT labeling of DA fibers and TH-positive neurons of the SN (Fig. 9). Thus, even in truly aged mice, a D_3-preferring agonist is able to reduce the impact of MPTP on multiple markers of DA neuron neurotoxicity. Importantly, the agonist was given before and during the MPTP treatment, but not for the subsequent 2-week recovery period. Thus, PPX could not be blocking the effects of MPTP by reducing the impact on disruption of the mitochondria (Cochiolo et al., 2000) or lipid peroxidation and oxidative stress (Zou et al., 2000) that occurs subsequent to MPTP conversion to MPP+ and uptake into the DA neurons. PPX might be inducing events that are protective for the neurons, as it has been shown to elevate the anti-apoptotic protein Bcl-2 in vivo (Takata et al., 2000) and in vitro (Kitamura et al., 1998).

The mechanisms by which direct DA agonists may provide neuroprotection are varied, and probably include their free-radical scavenging properties (Cassarino et al., 1998; Grunblatt et al., 1999b; Kitamura et al., 1998; Ling et al., 1998). Recent studies have extended these findings to in vitro systems by establishing that talipexole and PPX can prevent neurotoxicity produced by L-dopa in mesencephalic cultures (Carvey et al., 1997a; Ling et al., 1999) and by MPP+ in the neuroblastoma SH-SY5Y cell line (Kitamura et al., 1998). Carvey and associates have provided valuable evidence that it is the D_3 receptor that

mediates the effects of these DA agonists. The ability of PPX to inhibit DA neuron loss produced by L-dopa in mesencephalic tissue cultures is dependent on the generation of a factor with "growth promoting activity" released into the media (Carvey et al., 1997a). The source of this factor was unlikely to be in glia or non-DAergic neurons (Carvey & Ling, 1997). The protection was not induced by a D_2 agonist, and the neuroprotective effect of PPX was attenuated by a D_3-preferring antagonist and enhanced by D_3-preferring agonists (Carvey et al., 1997b; Ling et al., 1999). In contrast, another group has reported that attenuation of L-dopa-induced neurotoxicity in mesencephalic tissue cultures is D_2 receptor-mediated (Takashima et al., 1999). L-dopa induced neurotoxicity may not be as effective a model for PD as MPTP and its active metabolite MPP+ (Koshimura et al., 2000; Murer et al., 1998). However, ropinirole has also been shown to be neuroprotective against the intracerebral administration of 6-OHDA in mice, and this effect was blocked by the D_2-preferring antagonist sulpiride (Iida et al., 1999). Thus, it remains unclear as to whether these neuroprotective effects of DA agonists are through a D_2 or D_3 receptor, or both.

Extension of this research to the catecholaminergic neuroblastoma SH-SY5Y cell line also determined that pretreatment with the DA agonists bromocriptine, talipexole, and PPX significantly reduced MPP+ neurotoxicity (Kitamura et al., 1998). However, it was shown that blockade of DA receptors with either D_2-selective or non-selective compounds did not inhibit the neuroprotective effects of talipexole or PPX with the SH-SY5Y cells. It cannot only be due to the free radical scavenging properties of these compounds, as acute treatment with talipexole is less effective than pretreatment, and it has been shown that talipexole and PPX can elevate intracellular levels of Bcl-2. To test if the lack of DA receptor-mediated neuroprotective effects of D_2/D_3 agonists with the neuroblastoma SH-SY5Y cell line reflected properties of the undifferentiated SH-SY5Y cells, we examined this cell line after treatment with factors that differentiate these cells to a phenotypically mature DAergic cell type (Presgraves et al., 1999). With appropriate differentiation conditions, SH-SY5Y cells exhibit high levels of DAT, vesicular monoamine transporter, TH, D_2/D_3 receptors, and their G-proteins. Under these conditions, we were able to demonstrate that (1) PPX is neuroprotective with pretreatment; (2) the neuroprotection is not due to its antioxidant properties; (3) neuroprotection is not through regulation of DAT; and (4) neuroprotection occurs via D_2/D_3 receptor-mediated mechanisms. This suggests that D_3-preferring agonists may provide neuroprotection via induction of neuroprotective factors. Potential candidate neuroprotective factors include the neurotrophin BDNF and anti-apoptotic protein Bcl-2. The DA antagonist haloperidol reduces the levels of the neurotrophin BDNF, and can produce damage to DA neurons (Dawson et al., 2001; Levinson et al., 1998). BDNF is important for proper development of the mesostriatal DA system (Dluzen et al., 1999) and provides neuroprotection against MPTP-induced DA cell death (Pearce et al., 1999). While it has not been shown that PPX elevates levels of BDNF, there is evidence that Bcl-2 plays an essential role in the BDNF survival response of cultured neurons (Allsopp et al., 1995). The ability of PPX to elevate Bcl-2 (Takata et al., 2000) may potentiate the effects of BDNF on protection of DA neurons. The mechanism(s) by which PPX elevates Bcl-2 is unknown, and it would be important to identify if it is D_3 receptor-mediated. Neuroprotection by ropinirole against the effects of 6-OHDA in mice has been shown to be dependent on the elevation of the natural antioxidants glutathione, catalase, and superoxide dismutase (Iida et al., 1999). Therefore, it is likely that D_3-preferring agonists have a number of mechanisms through which neuroprotection could be induced, and this would add to their value as antiparkinsonian agents (Bennett & Piercey, 1999).

7. Conclusions

The original cloning of the D_3 receptor, the subsequent discovery of its pharmacological similarity to the D_2 receptors, and its higher expression in the limbic region of the striatum (Bouthenet et al., 1991; Sokoloff et al., 1990), allowed for its consideration as a potential target for antipsychotic drugs. The rationale for the therapeutic utility of the D_3 receptor has only been enhanced over the last decade. Some common behavioral functions of the mesolimbic DA D_3 receptor may be surmised based on the study of this receptor subtype in pathologic conditions (Fig. 1). Plasticity of the D_3 receptor in the mesolimbic DA system may represent the molecular basis for the common behavioral features observed in different disorders, such as PD, schizophrenia, or sensitization to psychostimulants. Elevations of the D_3 receptor occur in schizophrenia and in experimental conditions of hyperdopaminergic tone (Fig. 1B), which also may occur with L-dopa-induced dyskinesias in PD. Thus, D_3 receptor antagonists could prove to be effective in the treatment of schizophrenia, psychostimulant drug abuse, and drug-induced dyskinesias, without producing other extrapyramidal disorders. On the other hand, behavioral abnormalities in schizophrenia and PD may relate to opposite changes in the regulation of D_3 receptors. Such symptoms as depression, amotivation, and bradykinesia are frequently associated with PD (Starkstein et al., 1998; Vingerhoets et al., 1997). Reduced mesolimbic DA activity, partly due to a loss of D_3 receptors in PD, may underlie these behavioral symptoms. The effective antiparkinsonian D_3-preferring agonist PPX appears to have antidepressant effects (Maj et al., 1997; Corrigan et al., 2000), which may play a part in its effective antiparkinsonian effects (Piercey, 1998). Experimental models of PD suggest that D_3-preferring agonists do act through D_3 receptors to provide relief of

akinesia. We propose that adequate DA fiber innervation and DA stimulation is required to maintain appropriate levels of the D_3 receptor (Fig. 1A), damage to which produces a reduction in D_3 receptors (Fig. 1C) and contributes to a behavioral syndrome that includes amotivation/depression and motor retardation/bradykinesia. At early stages of PD, DA agonists are capable of restoring appropriate levels of the D_3 receptors, but this effect eventually declines in many PD cases. It will be important to determine if the clinical response to D_3-preferring agonists is maintained longer than with L-dopa and are particularly effective in reversing the reduction in D_3 receptor number. If so, antiparkinsonian agents that are D_3-preferring agonists, but with D_2 receptor actions may provide more effective relief of all symptoms asso-ciated with PD. Additionally, if D_3-preferring agonists are found to be neuroprotective in highly suitable models of PD, then this would provide a compelling rationale for introducing these drugs for the de novo treatment in PD. Development of more selective D_3 agonists and antagonists with improved bioavailability would greatly aid in the testing of these hypotheses.

Acknowledgments

The author is grateful for the support of an education grant from Pharmacia & Upjohn, Inc. and of grant USPHS NS 40669.

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Dopamine D_3 receptor antagonists as therapeutic agents

Jeffrey N. Joyce and Mark J. Millan

The behavioral and pathophysiological role of the dopamine D_3 receptor, which was deduced from anatomical, lesion and drug treatment studies in the ten years following cloning of the receptor, indicated that its functions differed from those of the D_2 receptor. There is increasingly strong evidence that D_3 receptor antagonists will be effective antipsychotic agents. In this regard, an amelioration of the negative and cognitive symptoms of schizophrenia holds the most promise for D_3 receptor antagonists, a concept currently under clinical evaluation. In addition, D_3 receptors could be involved in behavioral sensitization and the potential application of D_3 receptor antagonists in the treatment of drug abuse is undergoing intensive experimental investigation.

Dopamine exerts its actions through multiple classes of dopamine receptor, of which the dopamine D_1 and D_5 receptor families couple positively to adenylyl cyclase (adenylate cyclase), whereas the D2 family, which comprises D_2, D_3 and D_4 sites, couples negatively to this intracellular signal. Functional coupling of D_3 sites to G-proteins was initially considered less 'robust' than for their D_2 counterparts [1]. However, more recent studies have demonstrated efficient coupling of D_3 receptors via the $G_{\alpha i/o}$ subtype of G-protein to adenylate cyclase and several other transduction mechanisms: for example, mitogen-activated protein kinase and, possibly, K^+-channels [1,2]. Nonetheless, an important question has yet to be answered – which G-protein subtypes and signaling pathways are recruited by D_3 receptors *in vivo* in specific populations of neurons [2,3]? Guanine nucleotides have a lesser effect on the binding of agonists to D_3 compared with D_2 receptors, possibly enabling the D_3 receptor to retain high affinity for endogenous pools of dopamine [4]. It should be noted that D_3 receptors form heterodimers with D_2 receptors and adenosine A_{2A} receptors. These heterodimers have binding and coupling properties that are different from those of D_3 receptors [5,6]. The possible physiological and therapeutic significance of D_3 heterodimers is currently under evaluation; to date, they have been characterized exclusively with *in vitro* heterologous expression systems.

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Limbic system organization of D_3 receptors

The first evidence that selective D_3 receptor antagonists could be clinically relevant to schizophrenia came from the determination of their anatomical distribution in brain. In all species, the D_3 receptor is generally less abundant than the D_2 receptor, but the difference is particularly striking in the caudate putamen, where D_2 receptors are densest and D_3 receptors are poorly represented. D_3-binding sites and mRNA encoding D_3 receptors are concentrated in the nucleus accumbens (NACC), the limbic region of the striatum. This limited distribution of the D_3 receptor [7] suggests that its functions are primarily related to the mesolimbic, rather than the nigrostriatal, dopaminergic system [8]. The majority of D_3 receptors are expressed on cell bodies of the NACC and

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its terminals in the ventral pallidum and substantia nigra (SN) pars reticulata. That is, postsynaptic to dopaminergic terminals.

Presynaptic localization and function of D_3 receptors

One important issue has been whether D_3 autoreceptors, as well as D_2 autoreceptors, are expressed by dopaminergic neurons of the ventral tegmental area (VTA) and SN. One study found that the vast majority of tyrosine hydroxylase-positive neurons of the SN and VTA are labeled by an antibody recognizing the D_3 receptor in rat brain [9] and most are labeled with a D_2 mRNA probe [10], which is consistent with D_2 and D_3 receptors existing on dopamine neurons. Although previous functional studies have been contradictory [4], recent research underpins a specific role of D_3 receptors in the regulation of extracellular dopamine levels, perhaps via an interaction with the dopamine transporter (DAT) [11,12]. This would not preclude a major and obligatory role of the D_2 autoreceptor, but suggests interesting physical interactions between D_3 receptors and DATs in the regulation of extracellular levels of dopamine.

In the human brain, the highest expression of D_3 receptors is in the ventral striatum (NACC and ventral putamen), but, unlike the rodent brain, the association areas of the caudate nucleus and putamen are also enriched with these receptors [7]. This indicates a role for the D_3 receptor in multiple actions of dopamine (Figures 1,2). Another important difference from the rodent brain that has functional implications is the probable co-expression of D_3 and D_2 mRNA in neurons of many regions (e.g. thalamus), in addition to dopaminergic neurons of the VTA and SN. This could facilitate interactions between D_2 and D_3 receptors, and even the formation of heterodimers with altered signaling properties [5].

Probes for evaluating the roles of D_3 versus D_2 receptors

Specific antisense probes directed against D_3 or D_2 sites and genetically modified mice lacking D_2 and/or D_3 receptors have proven useful in the characterization of D_3 receptor function [13]. Indeed, the use of a D_3 receptor mutant (knockout) to delineate the role of D_3 versus D_2 receptors in the effects of dopaminergic agonists suggests that D_3 agonists act similarly in wild-type versus D_3 receptor knockout mice for several parameters, such as induction of hypothermia [14–16]. Although this approach has certainly helped to identify functional roles of D_3 receptors, selective pharmacological agents remain indispensable as

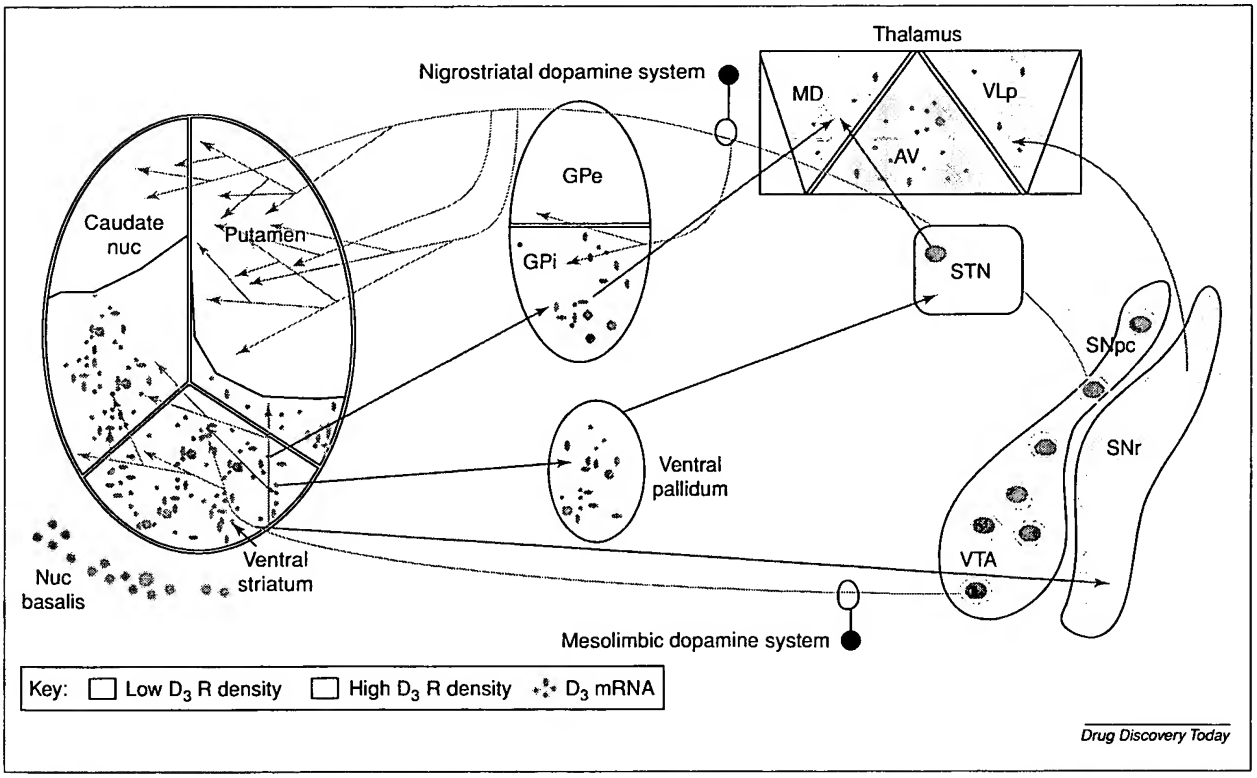

FIGURE 1

Neuronal localization of dopamine D_3 receptors within the limbic circuit. Schematic showing the localization of D_3 mRNA (grey dots) and D_3 receptor (yellow shading) in the subcortical regions of the human brain, primarily in the ventral striatum and its efferents. The grey arrows indicate the projections from one region to another. Note that projections from the ventral striatum to its efferents (ventral pallidum, globus pallidus internal and SN pars reticulata) and from these nuclei to thalamic nuclei are depicted ('limbic circuit' [4]). The dopamine innervation to the caudate and putamen (nigrostriatal) and to the ventral striatum (mesolimbic) are shown in blue arrows with their sites of origin in the SNpc and VTA, respectively, shown as grey circles surrounded by yellow (indicating D_3 mRNA). Abbreviations: GPe, globus pallidus external; GPi, globus pallidus internal; MD, medial dorsal; SNpc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; VLp, ventrolateral posterior.

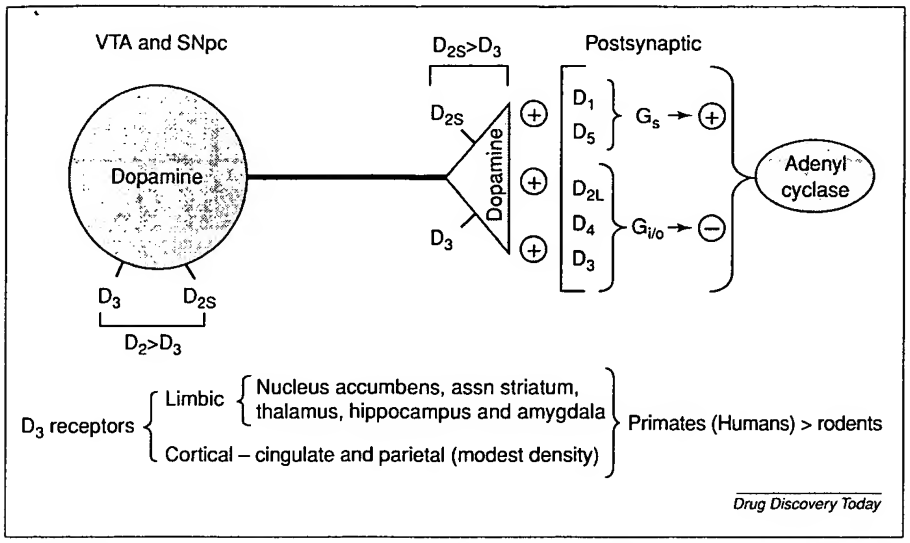

FIGURE 2

Cellular coupling of dopamine D_3 receptors compared with D_2 receptors and other classes of dopamine receptor. By analogy to dopamine D_2 receptors, of which short (S) and long (L) isoforms have been identified, D_3 receptors are localized postsynaptically and presynaptically to dopaminergic neurons. The density of D_3 autoreceptors is lower than that of their D_2 counterparts, but they both inhibit dopaminergic transmission. Dopamine receptors are coupled to various subtypes of G-proteins. Like other members of the D_2 receptor family, D_3 receptors inhibit adenylyl cyclase by recruitment of $G_{i/o}$. Other transduction mechanisms are also known but are not illustrated. In contrast to D_2 receptors, D_3 receptors are primarily localized in limbic and, to a lesser extent, cortical regions: their density in the caudate and putamen and other structures rich in D_2 receptors is lower. The level of cerebral D_3 receptors is higher in humans and other primates than in rodents. Abbreviation: assn striatum, association region of striatum.

experimental tools and, of course, as potential therapeutic agents.

Selective agonists that unambiguously differentiate actions mediated by D_3, compared with D_2, sites *in vivo* have not been described [4]. Notably, although 7-OH-DPAT and PD128,907 are widely used and instructive probes of D_3 receptor function, they are less selective than originally thought and potently activate D_2 receptors *in vivo* [4], which emphasizes the importance of selective antagonists at D_3 sites, the development of which has progressed systematically over the past ten years (Figure 3) [17,18]. Although the chemically related aminotetralin derivatives AJ76 and UH232, which pre-date cloning of D_3 receptors, show a marginal preference over D_2 sites, the first D_3 receptor antagonists to show significant selectivity for D_3 versus D_2 sites were U99194 and S14297 [19,20]. Unfortunately, U99194 has only low potency at D_3 receptors, compromising its utility *in vivo* [19,21]. Furthermore, S14297 has weak antagonist actions at muscarinic receptors and shows partial agonist properties at cloned, human D_3 receptors in specific coupling paradigms [3]. Drugs described subsequently include GR103,691, which is potent and selective but inactive at central D_3 receptors *in vivo*, and nafodotride, a benzamide with poor selectivity for D_3 over D_2 sites [19]. The next generation of improved D_3 receptor antagonists was initiated with GR218,231, a selective, potent and centrally active antagonist at D_3 versus D_2 sites; however, the use of GR218,231 has been minimal, reflecting its challenging synthesis [22,23]. By contrast, a more recently described selective D_3 receptor antagonist, SB277,011, has been used extensively (Figure 3) [24,25]. The equally selective, exceptionally potent and pure antagonist S33084 has also been thoroughly characterized *in vitro* and *in vivo* (Figure 3) [21–23,26]. The use of S33084 and SB277,011, in parallel with the preferential D_2 versus D_3 receptor antagonists L741,626 and S23199 [21,27], is currently the best approach for pharmacological identification of the roles of D_3 versus D_2 receptors in functional models.

Another interesting strategy to discern the significance of D_3 sites uses BP897 (Figure 3) [28]. This agent was described as a partial agonist at D_3 receptors and a less potent antagonist at D_2 sites, providing a 'double' affinity- and efficacy-based approach for distinguishing the roles of D_3 and D_2 sites [29]. However, several independent groups have questioned the agonist properties of BP897 at D_3 sites [26,30]. Furthermore, BP897 shows marked antagonist actions at other classes of monoamine receptor, such as 5-hydroxytryptamine 5-HT_{2A} and α_1-adrenoceptor [31]. Therefore, caution must be exercised in interpreting the actions of BP897, which should always be evaluated in interaction with genuinely selective D_3 antagonists.

Several selective D_3 receptor agents in development as therapeutic agents should also be mentioned (Figure 4). For example, AVE5997, which behaves as an antagonist, and PNU177,864, which has partial agonist actions at D_3 sites. However, literature data are lacking for these drugs, both of which appear to have been stopped because of concerns regarding safety [32]. In addition, A437,203 is currently undergoing Phase II trials, although clinical data are not yet available [27,33]. Another antagonist in Phase II for schizophrenia that should also be mentioned is S33138, which is an 'optimized' D_3 versus D_2 receptor antagonist [34–36].

Functional and therapeutic significance of D_3 receptors

D_3 receptor coupling to transduction mechanisms mimic those of D_2 receptors (Figure 2), but their cerebral distribution is extremely different. This indication of contrasting physiological and therapeutic significance is underpinned by functional studies suggesting that D_3 receptor antagonists could be useful in the management of several disorders [4,13,23]. In this regard, the most substantial database concerns schizophrenia, and thus the discussion of the clinical use of D_3 receptor antagonists focuses primarily on their potential utility as antipsychotic agents.

Possible role of D_3 receptors in the etiology of schizophrenia

Many investigators have proposed that the limbic regions of the striatum have an important role in the gating of disturbed 'limbic'-related neuronal processing in schizophrenia [37]. Because this region is, in turn, modulated by the mesolimbic dopaminergic system, where there is a high concentration of D_3 receptors, it has been proposed [8,38] that mesolimbic D_3 receptors mediate this 'gate'. Postmortem studies support this hypothesis in that they show that levels of D_3 receptors might be elevated in schizophrenics that are off antipsychotics [38], which is in contrast to D_2 receptors, which are elevated with antipsychotic treatment. The mechanism for the differential response of D_2 and D_3 receptors is not known, but specific possibilities can be eliminated. The elevation of D_3 receptors is unlikely to be as a result of the discontinuation of chronic antipsychotic treatment because D_3 receptor or mRNA levels are not elevated on chronic (9–11 months) antipsychotic treatment of rats, which is again in contrast to D_2 receptors [39]. However, postmortem studies are performed in patients who have received long-term drug treatment and who display a chronic disease state, conditions that are not matched precisely by any animal model, thus caution must be made in extrapolating from experimental work to humans.

The elevation of D_3 receptor levels might reflect a hyperdopaminergic state of the mesolimbic dopamine system that is well-documented in schizophrenia [40,41]. The hyperdopaminergic tone that is induced by pulsatile L-dopa administration to dopamine-depleted rats [42] or seen in DAT knockout mice is associated with elevated extracellular dopamine levels [43] and an overexpression of D_3 receptors without concomitant elevations in D_2 or D_1 receptors. Interestingly, D_3 receptors could be regulated by extrinsic non-dopaminergic signals, such as brain-derived neurotrophic factor (BDNF) [44,45]. BDNF activity is controlled by dopaminergic tone, with elevated dopamine resulting in enhanced BDNF release from corticostriatal fibers and an increase in D_3 receptor levels in the NACC [45]. Interestingly, antipsychotic treatment leads to a reduction of BDNF levels [46], but not to a reduction in the number of D_3 receptors. This could indicate a drastic reduction in levels of BDNF, which is normally released from corticostriatal and mesostriatal dopamine fibers, is required to cause a reduction in D_3 receptor number [44,45].

Additionally, a Ser9Gly polymorphism in the D_3 receptor (leading to more potent dopamine binding) could be associated with a higher risk of developing schizophrenia in some subgroups of patients [47,48]. However, some studies have been negative [49], indicating that gene polymorphisms in the D_3 receptor do not necessarily underlie an altered risk for developing schizophrenia. Nevertheless, recent research has reinforced the significance of such D_3 receptor polymorphisms, in particular in interaction with other genes [50–52].

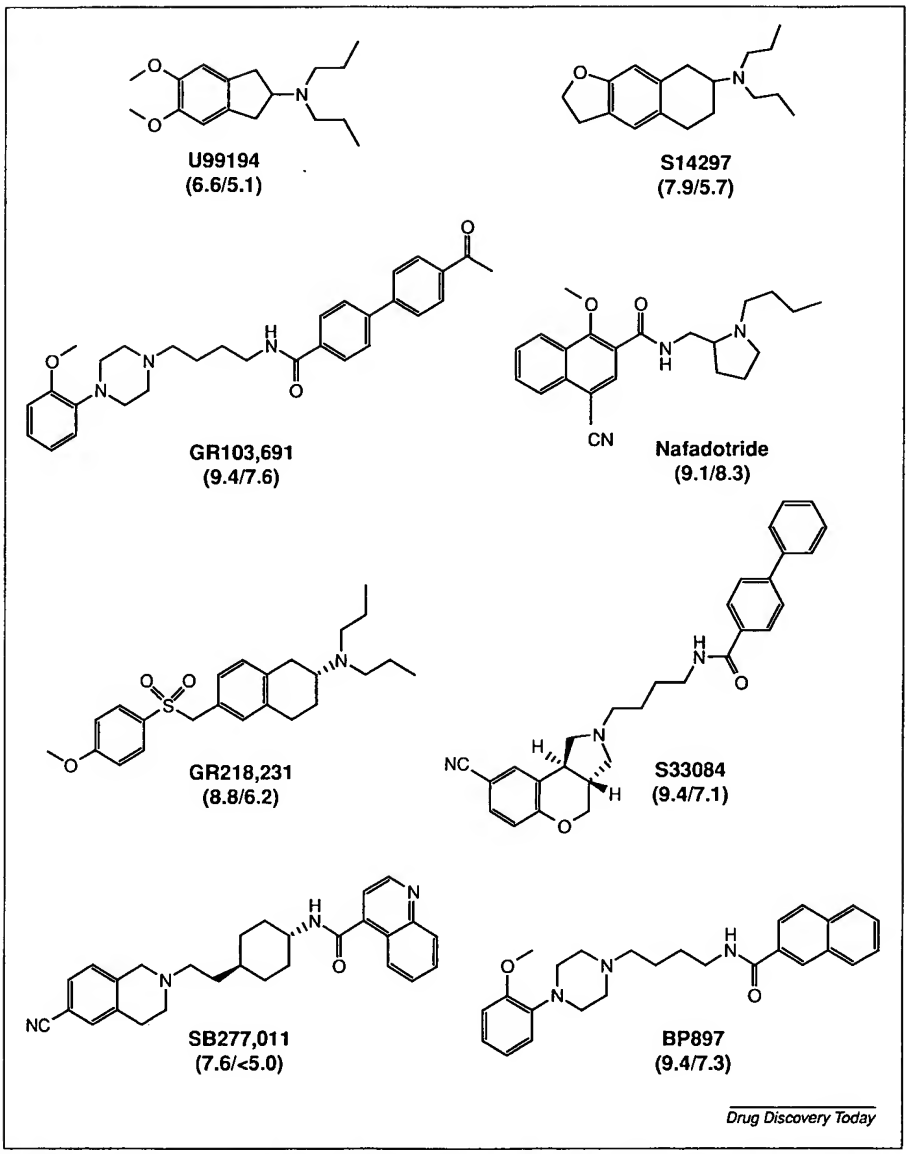

FIGURE 3

Chemical structures of drugs interacting preferentially with dopamine D_3 versus D_2 receptors. The values in parentheses are drug affinities (pK_i values given as D3/D2) for cloned, human D_3 and D_2 (long isoform) receptors transfected into Chinese hamster ovary cells and radiolabeled with [^{3}H]spiperone, which has comparable affinity for D_3 and D_2 sites. All results were obtained under identical conditions [19,22,23]. Note that all drugs behave as antagonists at cerebral D_3 and D_2 receptors *in vivo*, with the possible exception of BP897, which is a weak partial agonist at D_3 receptors and a pure antagonist at D_2 receptors.

Low extrapyramidal potential of D_3 receptor antagonists

Although the precise role of D_3 receptors in modulating motor behavior remains

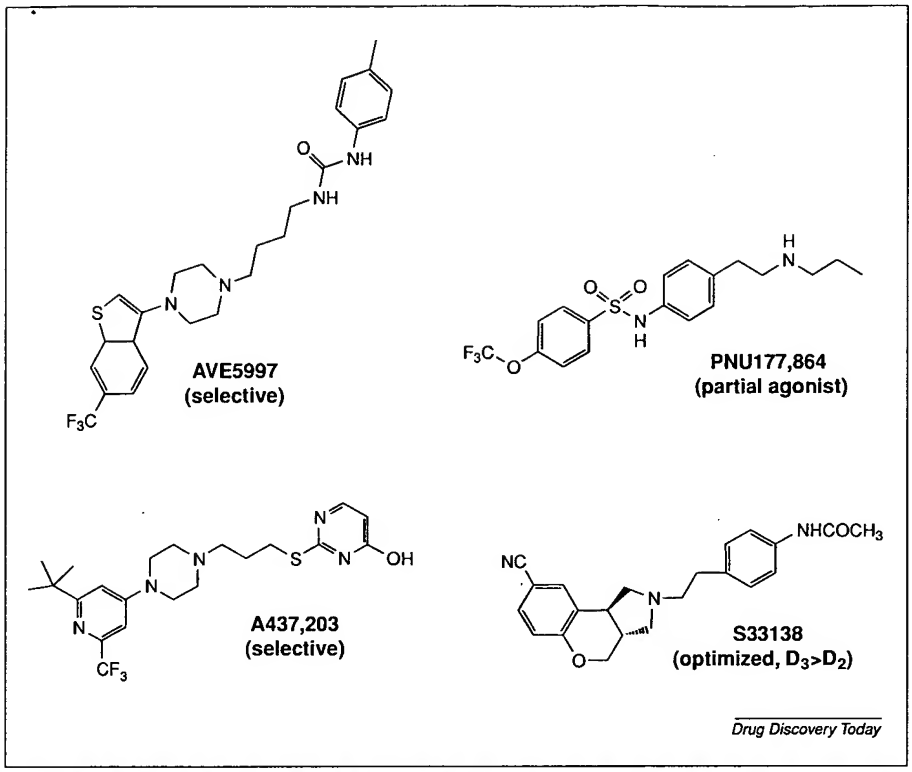

FIGURE 4

Chemical structures of drugs interacting preferentially with dopamine D_3 receptors that have been taken into the clinic for evaluation as potential antipsychotic agents. All drugs have been described as antagonists at cerebral D_3 and D_2 receptors, with the exception of PNU177,864, a partial agonist at D_3 sites. S33138 is not, in contrast to AVE5997 and A437,203, a highly selective antagonist at D_3 receptors but rather a preferential ('optimized') antagonist at D_3 versus D_2 receptors. Development of AVE5997 and PNU177,864 has apparently been discontinued, whereas S33138 and A437,203 are in Phase II trials for the treatment of schizophrenia.

unclear, and actions of drugs at both pre- and post-synaptic populations complicate analysis of data, the preponderance of evidence points to an inhibitory role [4,16,21]. Indeed, it is undisputed that selective inactivation of D_2 receptors (pharmacological or genetic) has a disruptive, inhibitory influence on motor function and underlies induction of an extrapyramidal (motor and endocrine) syndrome (EPS) by neuroleptics [21,25]. By contrast, selective blockade of D_3 receptors does not exert such effects. Recent studies in primates provide important insights into controversial rodent data in demonstrating that D_3 receptor antagonism, in a fashion opposite to D_2 receptor blockade, favors motor function [53]. Thus, a proportionally high degree of D_3 versus D_2 receptor blockade is predictive of a low EPS potential. However, it should be noted that a D_3 antagonist (nafadotride) and a D_3 partial agonist (BP897) can ameliorate the dyskinesias induced by subchronic treatment with L-dopa in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned Parkinsonian monkeys [54], suggesting that D_3 receptor partial agonists would have utility in treating L-dopa dyskinesias in Parkinson's disease patients. This effect of the D_3 receptor ligands has not been confirmed in two other studies [29,53], suggesting species differences in the effects of the drugs, or a lack of selectivity of BP897 and nafadotride [19].

Improved control of deficit symptoms by D_3 receptor antagonists

A useful classification of the symptoms of schizophrenia distinguishes positive (abnormal thoughts and perceptions) and negative (loss, or decrease, of normal functions) symptoms (Table 1) [55]. D_3 antagonists are not expected to elicit a marked EPS, which is important with regards to the treatment of negative symptoms (tend to be aggravated by the motor effects of neuroleptics). Depressed mood is common in schizophrenic patients and its presence can aggravate negative symptoms [53]. Whereas stimulation of D_2 receptors exerts a positive influence on depressed mood, their blockade elicits the opposite effect, perhaps contributing to the poor efficacy of antipsychotics having potent D_2 antagonist properties against negative symptoms [56,57]. However, there is no evidence that D_3 receptor blockade exerts a detrimental influence on mood, thus they lack this disadvantage [54,55]. Indeed, it is difficult to model negative symptoms in rodents but one approach is to examine the influence of drugs on social interaction, which is profoundly disrupted in patients. Under a variety of conditions, and using several different end-points, selective blockade of D_3, but not D_2, receptors enhances social interaction [24,33], observations that suggest that D_3 antagonists could improve negative symptoms in schizophrenic patients.

An alternative approach is provided by comparison of new drugs with the atypical antipsychotic clozapine, which shares characteristics of other atypicals (i.e. risperidone, olanzapine, quetiapine and ziprasidone), including a lack of motor side effects (e.g. EPS and tardive dyskinesia). Compared with other conventional antipsychotics (e.g. haloperidol), clozapine shows a higher therapeutic window, is more effective in moderating negative symptoms and elicits a distinctive and regionally selective pattern of influence on many genes in the CNS [58]. One such family of genes are the immediate early genes (IEG), including the protooncogenes c-*fos* and *fosB*, which, in turn, stimulate transcription factors responsible for adaptive changes in mature neurons [4]. The role of D_3 receptor blockade in the differential modulation of IEG responses by clozapine compared with haloperidol is controversial [59], but D_3 receptors are implicated in IEG modulation, in that clozapine failed to modify the expression of the IEG product *fosB* – Δ-FosB – in mice lacking D_3 receptors: furthermore,

TABLE 1

Positive and negative symptom profile of schizophrenia

Positive symptoms	Negative symptoms
Disordered thinking	Blunted affect
Delusions	Impaired attention
Hallucinations	Avolition
	Anhedonia
	Disrupted executive function

its actions were mimicked by selective D_3 receptor antagonists [60]. Thus, there could be an important D_3 receptor component in the therapeutic actions of clozapine.

Improved control of cognitive symptoms by D_3 receptor antagonists

In contrast to haloperidol, clozapine enhances cholinergic transmission in frontal cortex [61], an effect relevant to cognitive deficits because frontocortical cholinergic pathways have an important role in the modulation of attention, working memory and social memory, all of which are perturbed in schizophrenia [62–64]. Unfortunately, the direct muscarinic antagonist effects of clozapine compromise the benefits of an increase in acetylcholine release [65]. Thus, it is interesting to note that selective D_3 receptor antagonists (devoid of muscarinic effects) robustly enhance acetylcholine release in frontal cortex [66,67]. Parallel behavioral studies show that selective D_3 receptor antagonists also enhance cognitive function in models of working memory in rodents and relieve amnesic effects of scopolamine [68]. Studies in mice lacking D_3 receptors suggest that particular (although not all) facets of mnemonic function are also improved [69]. In contrast to D_3 sites, blockade or genetic inactivation of D_2 receptors neither enhances acetylcholine release nor improves cognitive performance [66,68,69]. Thus, D_3 receptor antagonism might improve cognitive deficits in schizophrenic patients that are poorly treated by currently available agents, including clozapine [70]. Their actions mimic the behavioral (procognitive) and neurochemical (enhanced acetylcholine release) actions of D_1 receptor agonists and would have the advantage of no long-term desensitization and a less generalized influence on, for example, motor behavior and the hypothalamic–pituitary adrenal axis [71]. However, there is a need for additional study of the precise influence of – short- and long-term – D_3 receptor blockade on cognitive function. Finally, one question that could be asked is – why do the currently available antipsychotic agents with D_3 receptor antagonist properties not robustly improve cognitive function? The most probable explanation is that their other pharmacological properties interfere with cognitive performance: notably, potent antagonist properties at muscarinic receptors; blockade of α_1-adrenoceptors and potent blockade of D_2 and/or D_1 receptors [63,72].

Paradoxically, the most troubling question is whether or not selective D_3 receptor antagonists will be effective in treating positive symptoms. Observations that their levels change in patients are not synonymous with the demonstration that their blockade will be effective [33]. A hypoactivity at *N*-methyl-D-aspartate glutamate receptors has been implicated in the induction of psychotic states. Accordingly, acute administration of low doses of noncompetitive antagonists at these sites, such as dizocilpine, phencyclidine or ketamine, increases locomotor activity in rodents [36,73]. Inhibition of their actions by haloperidol and other D_2- and D_3-like receptor antagonists is well demonstrated [74]. Antagonism or knockout of D_3 receptors also reduces dizocilpine-induced hyperactivity [75], but other results do not support these findings [23]. Moreover, selective D_3 receptor antagonists do not abrogate behavioral effects of psychostimulants (e.g. cocaine) and hallucinogens (e.g. mescaline) that also – albeit less closely – mimic particular aspects of schizophrenia [23,24]. Nevertheless, such models have, to a large extent, been developed to characterize existing classes of antipsychotic agent having D_2 and/or 5-HT_{2A} receptor antagonist properties. Thus, lack of activity in these models is not necessarily predictive of a lack of therapeutic efficacy.

Deficits in sensorimotor gating, assessed by prepulse inhibition (PPI) of the startle reflex, have been reported in schizophrenia, and might best correlate with positive symptoms [76]. Dopamine stimulant- or agonist (apomorphine)-induced disruptions of PPI are prevented by typical and atypical antipsychotics and current data indicate that disruption of PPI is mediated through D_2 receptors, but not D_3 or D_4 receptor subtypes [24,77]. However, in some behavioral models, evidence for antipsychotic efficacy of D_3 receptor antagonists has been observed. First, by analogy to deficits observed in schizophrenia, social isolation of young rats perturbs sensory gating (indicated by reduced PPI) and this behavioral change is reversed by a selective D_3 receptor antagonist [24]. Second, chronic treatment of rats with D_2 and D_3 receptor agonists provokes a progressive increase in locomotor activity that could be related to the enhanced function of mesolimbic dopaminergic pathways that is observed in schizophrenic patients [39]. This increase in locomotor activity can be attenuated by concurrent treatment with selective D_3 receptor antagonists [78] without these drugs inhibiting the acute motor responses to dopamine agonists [24]. Third, chronic administration of D_3 receptor antagonists consistently reduces the electrical activity of VTA-derived mesolimbic dopaminergic neurons [24,79]. Like clozapine [80], this action is selective, compared with SN-derived nigrostriatal neurons, which is in accordance with a low EPS potential of D_3 receptor antagonists. These observations suggest that chronic blockade of D_3 receptor antagonists could be associated with effects not necessarily seen on their acute antagonism, suggesting the need for further study of adaptive processes induced by long-term exposure to D_3 receptor

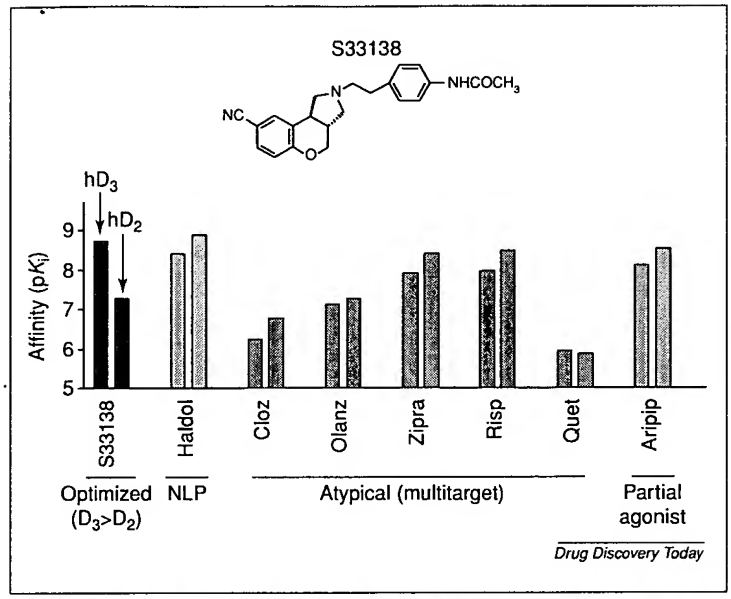

FIGURE 5

The novel agent S33138 preferentially interacts with dopamine D_3 rather than D_2 receptors. Drug affinities at cloned, human D_3 and D_2 (long isoform) receptors transfected into Chinese hamster ovary cells and radiolabeled with [^{3}H]spiperone were obtained under identical laboratory conditions. Binding data for S33138 are from [33], those for aripiprazole are unpublished and data for all other agents are from [87]. S33138 is the only drug to have higher affinity at D_3 sites than at D_2 sites. Abbreviations: aripip, aripiprazole (Abilify); cloz, clozapine (Clozaril); haldol, haloperidol (Haldol); NLP, neuroleptic; olanz, olanzapine (Zyprexa); PAG, partial agonist; quet, quetiapine (Seroquel); risp, risperidone (Risperdal); zipras, ziprasidone (Geodon).

antagonists, not only at the receptor itself but also at, for example, downstream signaling cascades [1,4,38].

A novel concept: 'optimized' antagonists at D_3 versus D_2 receptors

The persistent concern as to whether selective D_3 receptor blockade can relieve positive symptoms has engendered the concept of 'optimized' D_3 versus D_2 receptor antagonists, which is exemplified by the novel agent S33138 (currently in Phase II trials) [34,35]. This drug has preferential, but not absolute, selectivity for D_3 versus D_2 receptors (Figure 5). As such, it could be differentiated from other classes of agents: notably, from the neuroleptic haloperidol, from clozapine and other 'atypical' agents that have comparatively high affinity for serotonin (5-HT_{2A}) and adrenoceptor (α_1) versus D_2 receptors, and from aripiprazole, a multireceptorial drug that behaves as a partial agonist at D_2 and D_3 receptors [63]. At low concentrations, S33138 behaves as a pure antagonist at cloned human D_3 sites but, at higher doses, D_2 receptor blockade occurs. *In vivo* studies of the influence of S33138 on the motor actions of ropinirole in primates confirm that there is a ~20-fold dose-separation for doses blocking cerebral populations of D_2 versus D_3 receptors [34]. Ideally, S33138 will prove an effective agent at low doses. By contrast, if only cognitive and negative symptoms are improved at low doses, high doses can be administered to alleviate positive symptoms. Even at high doses, a satisfactory dose-separation of beneficial versus EPS effects should be achieved by virtue of its pronounced antagonism of D_3 receptors, an action favoring motor function. The preclinical profile of S33138 is consistent with these hypotheses: it enhances cognitive function and cortical acetylcholine release, increases social interaction and, at higher doses, is robustly active in models predictive of the control of positive symptoms without eliciting marked catalepsy [34]. Phase I investigations have confirmed the lack of motor side-effects of S33138 at single doses of up to 80 mg. Furthermore, imaging [positron emission tomography (PET)] studies in volunteers showed that S33138 (10–70 mg p.o., 1 h post-administration) was linearly displaced by a maximum of ~75% [^{11}C]raclopride binding to D_2 receptors (principally) in the striatum, indicating satisfactory CNS penetration (unpublished results). Because [^{11}C]raclopride principally binds to D_2 receptors, by extrapolation, doses required to occupy D_3 receptors should be substantially lower. Unfortunately, no selective PET ligands for D_3 receptors are currently available, which would permit a direct evaluation of the level of binding to D_3 receptors. Efficacy data from patients will be acquired over the next 1–2 years: such data should provide crucial clinical feedback concerning the genuine significance of D_3 receptors as targets for the management of psychotic states.

D_3 receptor antagonists in the treatment of drug abuse

By analogy to schizophrenia, elevations in limbic levels of D_3 receptors have been found in post-mortem studies of cocaine addicts. This is an interesting observation because cocaine addiction is a risk factor for schizophrenia and is likewise associated with a sensitization of mesolimbic dopaminergic pathways and excessive mesolimbic release of dopamine [81,82]. Reciprocally, schizophrenics show a high incidence of the abuse of cocaine and other drugs. In light of these findings, it is of interest that recent studies suggest a perhaps unique therapeutic utility for D_3 receptor antagonism for reducing cocaine and heroin addiction. SB277,011-A inhibits cocaine-seeking behavior, attenuates the cue-incentive properties of cocaine and blocks drug-seeking behavior specifically evoked by re-exposure to cocaine and, importantly, stress [83,84]. Similar findings were shown with opiates, nicotine and ethanol [85], suggesting a broader utility of D_3 receptor antagonism to attenuate drug taking. BP897 also attenuates the discriminative stimulus effects of cocaine in monkeys [86] and inhibits cocaine-seeking in rodents [28]. BP897 is a weak partial agonist at D_3 sites [30], thus their blockade potentially underlies these observations, which are analogous to those obtained with SB277,011-A and other selective D_3 receptor antagonists [56,83]. In view of the commonalities between cocaine abuse and schizophrenia, the notion of D_3 receptor antagonists as doubly active against both disorders is highly attractive.

Conclusions

Although it is 15 years since D_3 receptors were cloned, only over the past five years has their functional and potential therapeutic significance become clearer, as a consequence of the availability of selective antagonists and knockout mice. Selective antagonists are now entering clinical trials in schizophrenia, drug abuse (probably) and, it would be anticipated, cognitive disorders. The route has been long and difficult, but the next few years should at last provide concrete clinical feedback concerning the significance of D_3 receptors as targets for the treatment of CNS disorders.

Acknowledgements

We thank Gilbert Lavielle for assistance with Figures 3 and 4 and Jean-Michel Rivet for logistical support. The continued support of the Sun Health Foundation is gratefully acknowledged.

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Psychopharmacology (2005) 179: 567–575
DOI 10.1007/s00213-004-2096-z

ORIGINAL INVESTIGATION

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Dopamine D_3 receptor antagonists improve the learning performance in memory-impaired rats

Received: 16 April 2004 / Accepted: 18 October 2004 / Published online: 24 December 2004

Abstract *Rationale:* The dopamine D_3 receptor has been extensively studied in animal models of drug abuse and psychosis; however, less is known on its possible role in cognitive functions. *Objectives:* This study investigated the effects of different D_3 antagonists and a partial agonist on spatial learning performance in a water labyrinth test. *Methods:* Rats had to swim through a labyrinth system by making correct directional turns at three choice-points. The number of errors was recorded in three daily trials for 3 days. *Results:* D_3 antagonists such as the highly selective SB-277011 (24 mg/kg p.o.) and RGH-1756 (1 mg/kg p.o.), the moderately selective U-99194A (12 mg/kg s.c.) and the selective partial D_3 agonist BP-897 (1 mg/kg i.p.) all significantly attenuated the learning deficit caused by FG-7142. Against scopolamine-induced amnesia, SB-277011 (24 mg/kg p.o.) was equally potent in showing protective efficacy; however, two times higher dose levels of U-99194A (24 mg/kg s.c.) and RGH-1756 (2 mg/kg p.o.) were required to attenuate the scopolamine-induced impairment. In contrast to the full antagonists, against scopolamine-induced amnesia, the partial agonist BP-897 (2 mg/kg i.p.) was inactive, even at the two times higher dose level. *Conclusions:* These data suggest that dopamine D_3 receptor antagonists possess cognition-enhancing activity which may be of benefit in the treatment of cognitive dysfunction associated with several psychiatric disorders.

Keywords Dopamine D_3 · Memory · Water labyrinth · Scopolamine · FG-7142 · Rat

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Introduction

The dopamine D_3 receptor, which belongs to the D_2-like dopamine receptor subfamily and was cloned more than a decade ago (Sokoloff et al. 1990), is largely expressed in the limbic system including the islands of Calleja, the nucleus accumbens and the olfactory tubercles (Sokoloff et al. 1990; Murray et al. 1994). The presence of D_3 receptors in projection regions of the mesolimbocortical system suggests a potential role of these receptors in reinforcement processes, emotion and cognitive functions. Accordingly, the dopamine D_3 receptor has long been considered as a promising therapeutic target in the treatment of drug abuse and schizophrenia (Le Foll et al. 2000; Schwartz et al. 2000). However, the possibility of using dopamine D_3 receptor ligands in the treatment of cognitive disorders has received little attention.

It has been well documented that mesolimbic–mesocortical dopamine plays an important role in learning and memory. A series of studies has demonstrated the participation of dopamine D_1- and D_2-like receptors in cognitive functions (Arnsten et al. 1995; Seamans et al. 1998; Wilkerson and Levin 1999). Furthermore, the possibility of complex interactions between dopaminergic and cholinergic systems, mediated by D_1- and/or D_2-like receptors, has been suggested in learning and memory processes (Levin et al. 1990; Gasbarri et al. 1993; Hersi et al. 1995; Umegaki et al. 2001).

On the other hand, the D_2 ligands used in these studies also show considerable binding potential to the dopamine D_3 receptor so their actions may involve D_3-mediated components. However, only a few studies have been conducted addressing the contribution of D_3 receptors in cognition. 7-OH-DPAT (R(+)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin), a moderately selective D_3 agonist, impaired passive avoidance performance in mice (Ukai et al. 1997) and produced disturbances in an object discrimination task in marmosets (Smith et al. 1999). Since neither the dopamine D_1 receptor antagonist SCH-23390 nor the D_2 antagonist (−)-sulpiride attenuated the amnestic effect of 7-OH-DPAT (Ukai et al. 1997; Smith et al.

1999), while the dopamine D_2/D_3 receptor antagonist raclopride alleviated the memory impairment induced by the compound (Smith et al. 1999), it was assumed to be a D_3 receptor-mediated action. The relatively selective D_3 antagonist nafadotride antagonised the scopolamine-induced memory deficit and enhanced the improving effect of the D_2-like receptor agonist quinpirole in a passive avoidance paradigm in rats (Sigala et al. 1997). These results have drawn attention to the role of dopamine D_3 receptors in memory processes. However, for a better understanding of the function of the D_3 receptor, studies using more selective D_3 ligands are needed. Furthermore, the studies need to be extended to other experimental models of learning processes in order to collect further evidence for the role of the D_3 receptor in cognition. Since most available compounds lack appropriate functional selectivity, research to clarify the functional relevance of the dopamine D_3 receptor requires new ligands with high affinity for the D_3 receptor and high selectivity over other receptors.

The aim of the present work was to investigate the effect of different dopamine D_3 receptor ligands on learning performance in rats. The influence of the D_3 ligands on spatial memory was investigated in a water labyrinth test reported earlier (Paróczai et al. 1998).

Aside from investigating the effects of compounds on the normal learning process, their activity was also examined in experimental amnesia models. Memory impairment was induced by scopolamine, a widely used anticholinergic amnestic agent, and by FG-7142 (*N*-methyl-β-carboline-3-carboxamide), a benzodiazepine inverse agonist. In addition to its anxiogenic properties, FG-7142 has been shown to disrupt learning performance in several paradigms (Murphy et al. 1996a,b, 1997; Ninan and Kulkarni 1999). The efficacy of typical and atypical antipsychotic compounds in attenuating the effects of FG-7142 (Murphy et al. 1996a, 1997; Ninan and Kulkarni 1999; Moore et al. 1999) suggests that FG-7142-induced learning impairment may be an appropriate model of cortical dysfunction, characteristic of schizophrenia. It is also relevant for studying the memory-improving activity of D_3 receptor antagonist ligands.

The following dopamine D_3 receptor ligands were tested in the water-labyrinth paradigm: the partial agonist BP-897 (*N*-[4-(4-(2-methoxy-phenyl)piperazinyl)butyl]-2-naphthamide); the antagonists SB-277011 (*trans-N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide) and U-99194A (5,6-dimethoxy-2-(di-*n*-propyl-amino)indan). In addition, RGH-1756 (6-[4-[4-[4-(2-methoxyphenyl)piperazin-1-yl]butoxy] phenyl]imidazo[2,1-*b*]thiazole), a selective D_3 receptor ligand developed by Gedeon Richter (Laszlovszky et al. 2001), was studied. RGH-1756 appeared to be an antagonist at the D_3 receptor (Horváth et al. 2001). The molecules studied possess various affinities and selectivities for the dopamine D_3 receptor (see Table 1).

Table 1 Affinity and selectivity for dopamine D_2 and D_3 receptors of the various ligands tested

Compound	D_2 K_i (nM)	D_3	D_2/D_3 ratio	Source
BP-897	61	0.92	66	Pilla et al. 1999
U-99194A	2,281	223	10	Audinot et al. 1998
SB-277011	1,047	11	93	Reavill et al. 2000
RGH-1756	12.2	0.12	102	Horváth et al. 2001

Materials and methods

Subjects

Male Wistar rats obtained from LATI, Hungary, weighing 180–200 g, were used in the experiments. The animals, housed five per cage, were kept at 24±2°C, on a 12:12-h light/dark cycle (lights on at 6:00 AM) in a laboratory animal care unit. The rats were given commercial pellet rat–mouse feed (ssniff R/M+H; Spezialdiäten GmbH, Germany), autoclaved at 105°C, and tap water, ad lib. All the procedures carried out on animals had been approved by the local ethics committee and conformed to the rules and principles of the 86/609/EEC Directive.

Apparatus

Experiments were performed in a three-choice-point water labyrinth (Paróczai et al. 1998). The water tank, made from rustproof metal, was 1 m long, 60 cm wide and 60 cm deep. The labyrinth system was constructed with removable vertical metal plates. The pool was filled with water of 24±2°C to a depth of 30 cm. The animals had to manoeuvre through three choice-points along the longer axis of the tank in order to reach a platform which allowed them to escape from the water. The escape platform (10×7 cm) with its top surface raised 0.5 cm above the level of the water was placed in the corner of the tank furthest from the start point.

Procedure

The procedure for testing water-labyrinth acquisition process was carried out on 4 or 5 consecutive days.

Adaptation (day 1). In order to get the animals to adapt to the test environment, the metal plates constituting the labyrinth system were removed from the water and rats were conditioned three times to swim in the tank from the start point to the platform. Animals were left on the platform for 20 s and they were allowed to rest in their cage for 30 min between each swim.

Training procedure (days 2–4; occasionally days 2–5, when work arrangements allowed learning performance to be followed by an additional day). One daily session consisting of three trials was performed. On the training

days the labyrinth system was in place. Rats were placed into the water at the start point of the labyrinth system and they had to reach the escape platform. The animals spent 20 s on the platform, and they were then placed into a common cage accommodating no more than five animals (always home-cage mates) for ~25-min rest periods between trials. The number of directional turning errors was measured as a variable reflecting learning performance. An error was defined as swimming through a choice-point in the direction which did not allow the animal to reach the platform (blind alley). When the rat made an error it was allowed to swim back to the choice-point and try again. However, once a rat swam over a choice-point in the correct direction (leading out of the labyrinth), the way back was manually closed by a metal plate. Swimming time (the time interval from the entry into the labyrinth until the exit from the water) could not exceed 5 min for any trial. If the rat did not find the platform during this period, it was assisted to the end of the labyrinth by the experimenter and the number of errors was recorded as 12. This arbitrary value is high enough to express the fact that the animal was not able to perform the task within the limit time of a given trial. It exceeds the highest number of errors committed by naive rats while still accomplishing the labyrinth. On rare occasions, an animal committed more than 12 errors during the limit time, and in this case the actual value was recorded. Occasionally, some of the rats hung on to the metal plates of the labyrinth system and remained in this position during the trial period. At the end of the trial these rats were also guided to the escape platform by the experimenter. However, when a rat consistently adopted this strategy in more than 75% of all the trials, it was excluded from the experiment.

Three types of experimental design using separate groups of animals were applied in this study. (1) Effects of all the D_3 ligands were investigated on the memory impairment elicited by amnestic agents. These experiments included a solvent control, a memory-impaired group and an impaired group treated with a dopamine D_3 receptor antagonist/partial agonist ligand. The learning deficit was induced either by FG-7142 (20 mg/kg) or scopolamine (3 mg/kg). The amnestic agents were injected i.p. 30 min prior to the first daily trial. (2) SB-277011, U-99194A and BP-897 were also tested alone, i.e. without impairing agents, using the lowest dose of each compound investigated in the impaired learning paradigm. In these cases the experiment involved only solvent control and drug-treated groups. (3) The effect of RGH-1756 on normal learning was studied in parallel to the measurement of its influence on the learning disturbance produced by FG-7142. (See Table 2 for the design of each experiment.) There were ten animals in each experimental group. Dosages, routes, and pre-treatment times with the solutions/suspensions of the receptor ligands are summarised in Table 3. The D_3 ligands were studied at doses where the compounds—based on results from the original publications—did not show D_2 receptor antagonist effects (Waters et al. 1993; Pilla et al. 1999; Reavill et al. 2000 and our own unpublished results for U-99194A, BP-897, SB 277011 and RGH-1756, respectively).

Table 3 Dosing parameters of the compounds used in the study

Compound	Dose (mg/kg)	Route	Pre-treatment time (min)	Solution
BP-897	1.0	i.p.	30	5% Tween-80
U-99194A	12	s.c.	45	Distilled water
SB-277011	24	p.o.	30	5% Tween-80
RGH-1756	1.0	p.o.	60	Distilled water

Table 2 Summarised treatment regime used in the study

Test compound	Experiment number	Experimental groups			
		Vehicle(s)	Compound alone	Impairing agent	Impairment+compound
BP-897	1	+	BP-897 1 mg/kg i.p.	–	–
	2	+	–	FG–7142	BP-897 1 mg/kg i.p.+FG
	3	+	–	Scopolamine	BP-897 1 mg/kg i.p.+scopolamine
	4	+	–	Scopolamine	BP-897 2 mg/kg i.p.+scopolamine
U-99194A	5	+	U-99194A 12 mg/kg s.c.	–	–
	6	+	–	FG–7142	U-99194A 12 mg/kg s.c.+FG
	7	+	–	Scopolamine	U-99194A 12 mg/kg s.c.+scopolamine
	8	+	–	Scopolamine	U-99194A 24 mg/kg s.c.+scopolamine
SB-277011	9	+	SB-277011 24 mg/kg p.o.	–	–
	10	+	–	FG–7142	SB-277011 24 mg/kg p.o.+FG
	11	+	–	Scopolamine	SB-277011 24 mg/kg p.o.+scopolamine
RGH-1756	12	+	RGH-1756 1 mg/kg p.o.	FG-7142	RGH-1756 1 mg/kg p.o.+FG
	13	+	–	Scopolamine	RGH-1756 1 mg/kg p.o.+scopolamine
	14	+	–	Scopolamine	RGH-1756 2 mg/kg p.o.+scopolamine
	15	+	–	Scopolamine	RGH-1756 2 mg/kg p.o.+scopolamine

Data analysis

Individual values (number of errors) were averaged across the three daily trials, then group means were calculated from these daily individual data. Results are given as mean error±SEM of groups for each daily session. Statistical comparisons between parameters of each group were made by ANOVA (two-way repeated-measures analysis of variance) using 'groups' as the independent between-groups factor and 'days' as the repeated-measures factor. Post-hoc comparisons (Duncan test) were performed in case of a significant between-groups effect or a significant interaction between the independent and the repeated-measures factors.

The percent reversal by the compounds of the amnesia was calculated from the group means of pooled errors for all the trials in the training days using the formula:

$$\% = \frac{N_{err} \text{ of amnestic} - N_{err} \text{ of compound}}{N_{err} \text{ of amnestic} - N_{err} \text{ of control}} \times 100.$$

Drugs

SB-277011, BP-897, RGH-1756 and FG-7142 were synthesised at Gedeon Richter. U-99194A was obtained from RBI (Natick, MA, USA) and scopolamine HBr from Sigma (St. Louis MO, USA).

Results

In all experiments the control animals showed a marked improvement in the water-labyrinth acquisition: the number of directional turning errors (Figs. 1, 2, 3, 4) decreased considerably from day to day. The numerical data of FG-7142- or scopolamine-induced amnesia experiments are shown in Table 4.

The amnestic agents, scopolamine (3 mg/kg i.p.) and FG-7142 (20 mg/kg i.p.), disrupted the normal learning process, resulting in a significant increase in the number of errors. The memory deficit induced by scopolamine proved to be massive, frequently appearing on day 1, while FG-7142 exerted a mild to moderate impairing effect on the learning performance which became manifest from the second training day (Figs. 1, 2, 3, 4).

The partial D_3 receptor agonist BP-897, at a dose of 1 mg/kg i.p., significantly alleviated the FG-7142-induced memory impairment, as indicated by a decrease in the number of errors (Fig. 1a); however, even at a higher dose (2 mg/kg i.p.), the compound was inactive against scopolamine (Fig. 1b and c).

U-99194A, at a dose of 12 mg/kg s.c., significantly improved the learning deficit caused by FG-7142. The ligand practically reversed the increase in the number of errors (Fig. 2a). On the other hand, U-99194A given in the same dose failed to inhibit the amnestic effect of scopolamine (Fig. 2b); however, at twice the dose (24 mg/kg s.c) the compound reversed the scopolamine amnesia (Fig. 2c).

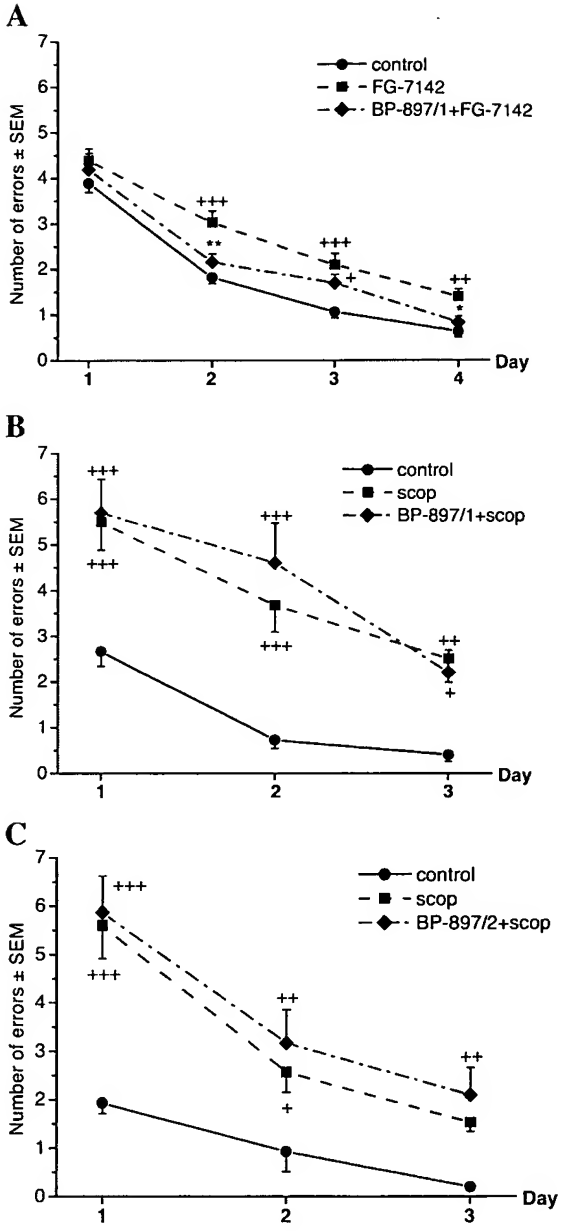

Fig. 1 Effect of BP-897 on the water-labyrinth acquisition in memory-impaired rats (n=10 animals per groups). Animals performed three daily trials; values shown are group means±SEM for all three trials in a day. F and p values relate to ANOVA 'groups' effects. a BP-897 (1 mg/kg i.p.) produced an alleviation in FG-7142-induced impairment [$F(2,27)=8.46$, $p<0.01$]. b, c BP-897 (1 and 2 mg/kg i.p.) failed to counteract the scopolamine amnesia [$F(2,27)=7.42$, $p<0.01$] and [$F(2,27)=7.645$, $p<0.01$, respectively]. +$p<0.05$, ++$p<0.01$, +++$p<0.001$ compared to control group; *$p<0.05$, **$p<0.01$ compared to memory-impaired group (post-hoc Duncan test)

At an oral dose of 24 mg/kg, SB-277011 attenuated the FG-7142-induced cognitive impairment (Fig. 3a). The effect was statistically significant only on day 2, when the disrupting effect of FG-7142 was at its greatest compared

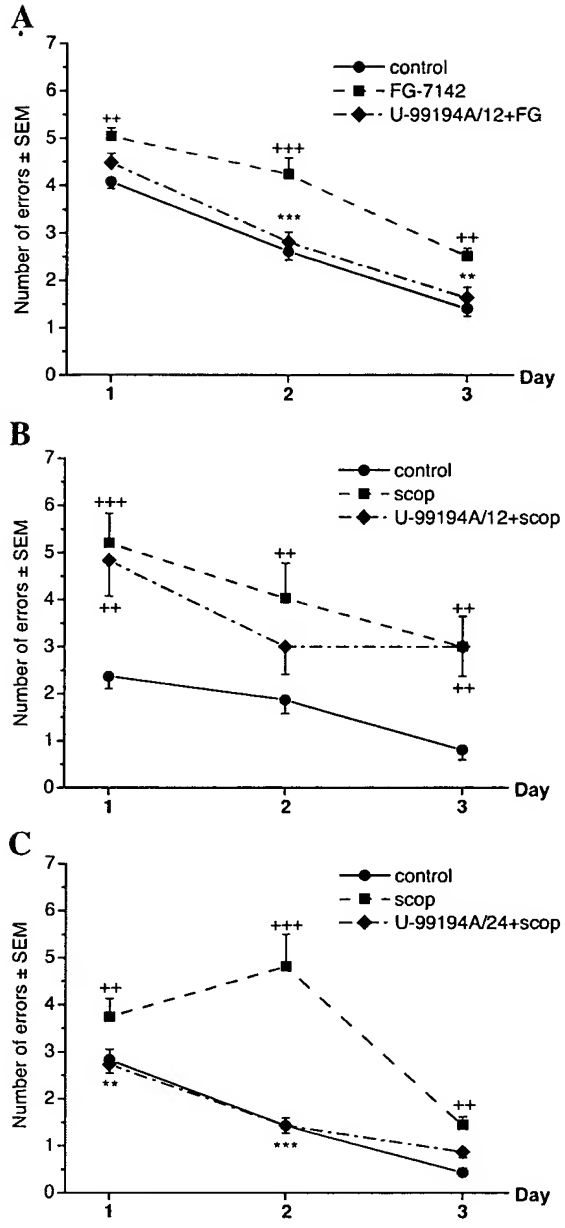

Fig. 2 Effect of U-99194A on the learning performance in (**a**) FG-7142-induced and (**b**, **c**) scopolamine-induced amnesia, n=10 animals per group. For details, see Fig. 1. **a** U-99194A (12 mg/kg s.c.) significantly restored the increase in the number of errors induced by FG-7142 [$F(2,27)=14.26$, $p<0.001$]. **b** U-99194A (12 mg/kg s.c.) did not show significant protective effect against scopolamine-induced amnesia [$F(2,27)=3.38$, $p<0.05$]. **c** U-99194A (24 mg/kg s.c.) reversed the scopolamine-produced learning deficit [$F(2,26)=27.44$, $p<0.001$]. ++$p<0.01$, +++$p<0.001$ compared to control group; **$p<0.01$, ***$p<0.001$ compared to memory-impaired group (post-hoc Duncan test)

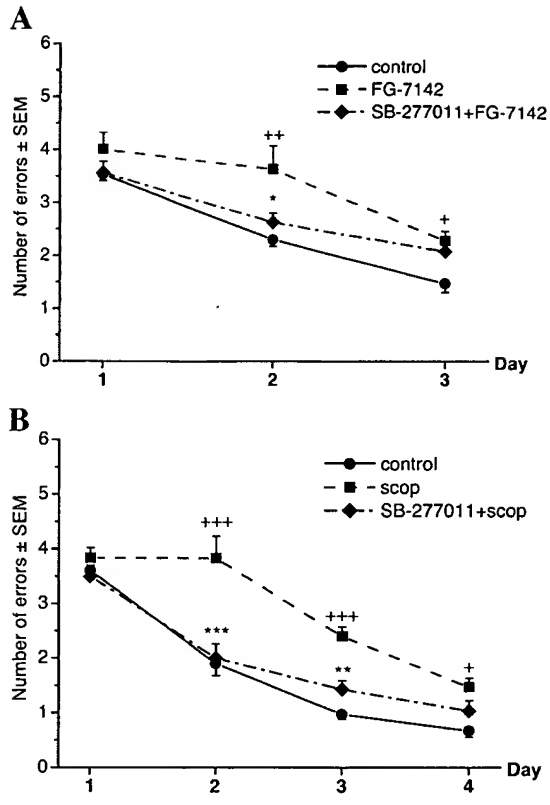

Fig. 3 Effect of SB-277011 (24 mg/kg p.o.) on the learning process of (**a**) FG-7142-impaired and (**b**) scopolamine-impaired rats, n=10 animals per group. For details, see Fig. 1. **a** SB-277011 moderately reduced the FG-7142-induced increase in the number of errors [$F(2,26)=4.34$, $p<0.05$]. **b** SB-277011 significantly decreased the elevated number of errors induced by scopolamine [$F(2,27)=18.44$, $p<0.001$]. +$p<0.05$, ++$p<0.01$, +++$p<0.001$ compared to control group; *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to memory-impaired group (post-hoc Duncan test)

to control. However, SB-277011 caused a marked decrease in the number of errors produced by scopolamine administration, reducing them almost to control level (Fig. 3b).

BP-897, U-99194A and SB-277011, given alone at doses of 1 mg/kg i.p., 12 mg/kg s.c. and 24 mg/kg p.o., respectively, did not influence the water-labyrinth performance (data not shown).

At a daily dose of 1 mg/kg p.o., RGH-1756 exerted a remarkable restorative effect on the impaired learning process of FG-7142-treated animals from the third experimental day (Fig. 4a). A single administration of RGH-1756 did not affect learning ability in unimpaired rats (Fig. 4a). Treatment with 1 mg/kg oral dose of RGH-1756 did not show a protective effect against scopolamine (Fig. 4b); however, by doubling the dose, the compound significantly improved the acquisition of the water-labyrinth task (Fig. 4c). In the case of the 2-mg/kg dose, two repeated experiments were performed due to the high variability of RGH-1756 effect on the third day in the first study. The pooled data obtained from two experiments are summarised in Fig. 4c.

Figure 5 presents the percent reversal of FG-7142- and scopolamine-induced amnesia produced by various dopamine D_3 receptor antagonists and a partial agonist (BP-897). All ligands exerted significant memory-improving activity against FG-7142; however, their effect proved to be different in scopolamine amnesia, where BP-897 was inactive, while U-99194A and RGH-1756 showed efficacy

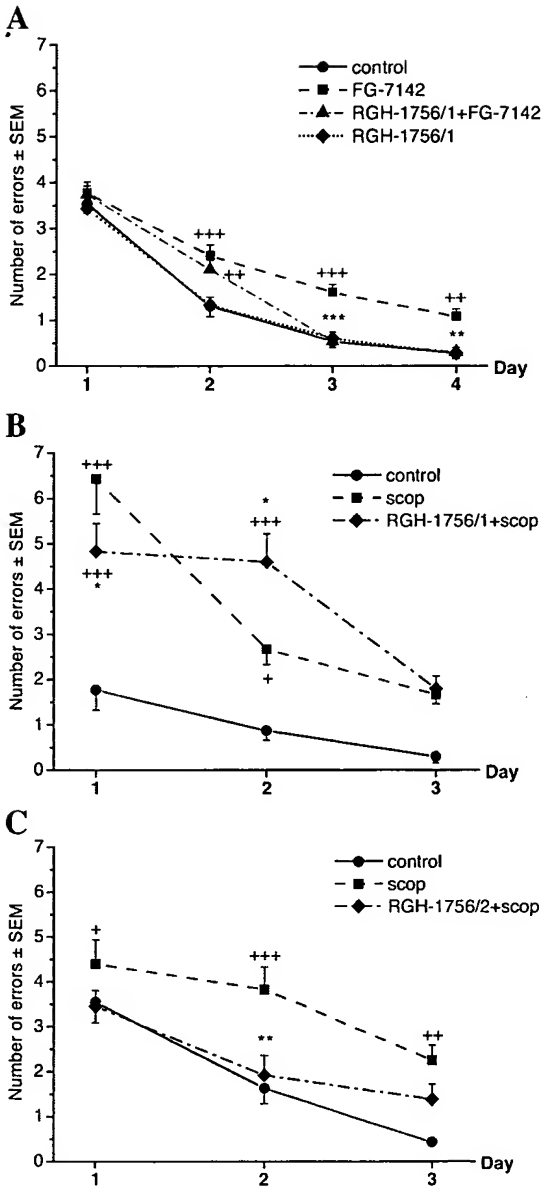

Fig. 4 Effect of RGH-1756 on the normal and FG-7142-impaired learning (**a**) and scopolamine-induced amnesia (**b**, **c**). For details, see Fig. 1. **a** RGH-1756 (1 mg/kg p.o.) time-dependently restored the learning deficit induced by FG-7142 (n=10 animals per group) [$F(3,36)$=6.63, p<0.01]. **b** RGH-1756 at a dose of 1 mg/kg p.o. (n=10 animals per group) was not active on scopolamine-induced amnesia [$F(2,27)$=16.23, p<0.001]. **c** The 2-mg/kg p.o. dose of RGH-1756 significantly improved the learning performance of scopolamine-impaired animals (n=20 animals per group) [$F(2,57)$ =3.99, p<0.05]. +p<0.05, ++p<0.01, +++p<0.001 compared to control group; *p<0.05, **p<0.01, ***p<0.001 compared to memory-impaired group (post-hoc Duncan test)

at twice the dose levels effective in FG-7142-induced impairment. At the same dose level, SB-277011 possessed greater protective activity against scopolamine-induced impairment than against that induced by FG-7142.

Table 4 Numerical data of FG-7142- or scopolamine-induced amnesia experiments

Type of study, drug/dose (mg/kg)+ impairment	Experimental groups											
	Vehicle				Impairing agent				Impairment+compound			
	Mean error±SEM				Mean error±SEM				Mean error±SEM			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
BP-897/1 i.p. + FG-7142	3.9±0.2	1.83±0.14	1.07±0.14	0.63±0.13	4.4±0.26	3.03±0.26	2.1±0.25	1.4±0.16	4.2±0.35	2.17±0.17	1.7±0.19	0.83±0.14
BP-897/1 i.p. + scopolamine	2.67±0.33	0.73±0.19	0.4±0.15	–	5.5±0.62	3.67±0.59	2.5±0.52	–	5.7±0.73	4.6±0.86	2.2±0.48	–
BP-897/2 i.p. + scopolamine	1.93±0.22	0.93±0.42	0.2±0.07	–	5.6±0.69	2.57±0.42	1.53±0.19	–	5.87±0.75	3.17±0.69	2.1±0.56	–
U-99194A/12 s.c. + FG-7142	4.07±0.15	2.6±0.18	1.4±0.17	–	5.03±0.17	4.23±0.34	2.5±0.16	–	4.47±0.2	2.8±0.21	1.63±0.22	–
U-99194A/12 s.c. + scopolamine	2.37±0.27	1.87±0.3	0.8±0.21	–	5.2±0.63	4.03±0.74	3.0±0.64	–	4.83±0.76	3.0±0.59	3.0±0.63	–
U-99194A/24 s.c. + scopolamine	2.83±0.22	1.43±0.16	0.43±0.09	–	3.74±0.39	4.81±0.69	1.44±0.17	–	2.73±0.19	1.43±0.16	0.87±0.12	–
SB-277011/24 p.o. + FG-7142	3.53±0.12	2.3±0.13	1.47±0.17	–	4.0±0.32	3.63±0.44	2.27±0.18	–	3.56±0.21	2.63±0.17	2.07±0.18	–
SB-277011/24 p.o. + scopolamine	3.6±0.17	1.9±0.22	0.97±0.09	0.67±0.12	3.83±0.19	3.83±0.4	2.4±0.17	1.47±0.16	3.5±0.2	2.0±0.26	1.43±0.16	1.03±0.19
RGH-1756/1 p.o. + FG-7142	3.53±0.21	1.3±0.22	0.53±0.13	0.27±0.12	3.77±0.24	2.4±0.24	1.6±0.18	1.07±0.17	3.73±0.19	2.1±0.2	0.53±0.1	0.3±0.1
RGH-1756/1 p.o. + scopolamine	1.77±0.44	0.87±0.21	0.3±0.14	–	6.43±0.77	2.67±0.34	1.67±0.21	–	4.83±0.62	4.6±0.62	1.8±0.28	–
RGH-1756/2 p.o. + scopolamine	3.55±0.46	1.63±0.35	0.43±0.09	–	4.4±0.54	3.83±0.5	2.25±0.34	–	3.45±0.36	1.92±0.44	1.38±0.34	–

Data are mean error±SEM of groups calculated from three trials for 3 or 4 days.

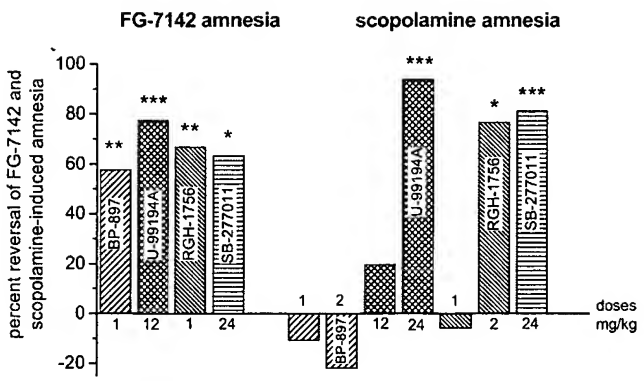

Fig. 5 Percent restoration effect of D_3 receptor antagonist/partial agonist ligands in FG-7142- and scopolamine-induced memory deficiency (calculated as described in Data analysis). $*p<0.05$, $**p<0.01$, $***p<0.001$, post-hoc Duncan test was performed on group means pooled over days. For results of ANOVA, see the legend of the appropriate figure. For routes of administration of the receptor ligands, see Table 3.

Discussion

The dopamine D_3 receptor antagonists (U-99194A, SB-277011 and RGH-1756) and partial agonist (BP-897), investigated alone in the water-labyrinth task, were without any influence on the normal cognitive processes. However, all D_3 ligands studied in our experiments showed memory-improving activity to some degree in amnesia models. The partial agonist BP-897 (1 mg/kg i.p.), and particularly the modestly selective D_3 antagonist U-99194A (12 mg/kg s.c.), potently and significantly reversed the learning disturbance caused by FG-7142. The highly selective SB-277011 (24 mg/kg p.o.) alleviated the memory impairment only on the second experimental day and similarly, RGH-1756 reversed the FG-7142-induced cognitive deficit from the third day of the water labyrinth test. SB-277011 was the only D_3 antagonist ligand that counteracted the memory impairment induced by scopolamine at the same dose as in the FG-7142 model. Both U-99194A and RGH-1756 at two times higher doses (24 mg/kg s.c. and 2 mg/kg p.o., respectively) significantly attenuated the cognitive deficit produced by scopolamine treatment. However, BP-897 even at a twofold dose (2 mg/kg i.p.) proved to be inactive in scopolamine amnesia.

The inactivity of D_3 receptor antagonist/partial agonist ligands on the normal learning process in non-impaired rats is in contrast with the memory-impairing effect of D_2 preferring antagonists (Wilkerson and Levin 1999; Umegaki et al. 2001; Myhrer 2003). It suggests that the doses selected in the present study did not target the D_2 receptor. On the other hand, as is generally the case with other cognition-enhancing drugs, the D_3 compounds did not facilitate normal acquisition, while they were active against amnestic agents.

Since the mechanisms underlying the learning impairment produced by each pharmacological manipulation are distinct, the protective effect of D_3 antagonists/partial agonists against FG-7142 or scopolamine appears also to be diverse. The behavioural effects of FG-7142 seem to be very complex in nature, involving activation of cholinergic (Sarter et al. 2001), glutamatergic (Karreman and Moghaddam 1996) and noradrenergic (Birnbaum et al. 1999) systems. For example, Moore et al. (1995) found that the compound strongly elevated the cortical acetylcholine efflux, which—according to the hypothesis of Sarter et al. (2001)—induces a hyperattentional state leading to impairment in cognitive/cortical processing, a defect seen in schizophrenics as well. However, Murphy et al. (1996a,b) demonstrated that FG-7142 induced an increase of dopamine release in the prefrontal cortex. The degree of impairment in prefrontal cortical-dependent spatial working memory functions correlated with the elevated level of dopamine turnover. This increase in dopaminergic transmission is suggested to be the critical neurochemical component of the spatial working memory disturbance produced by the drug. It is assumed that the normal cognitive function requires an optimal activation of the D_1 receptors in the prefrontal cortex (Williams and Goldman-Rakic 1995; Murphy et al. 1996a; Zahrt et al. 1997). Both decrease (Bubser and Schmidt 1990; Seamans et al. 1998) and increase (Zahrt et al. 1997) in stimulation of D1 receptors in the prefrontal cortex have been shown to produce cognitive impairment. The spatial working memory deficit observed in mice lacking D_2 and D_3 receptors was suggested to result from decreased prefrontal cortical D_1 receptor activation (Glickstein et al. 2002). However, FG-7142-induced learning deficit can be reversed not only by the selective dopamine D_1 antagonist SCH-23390, but by haloperidol, clozapine or olanzapine as well, and even the D_2/D_3 antagonist sulpiride is active in this respect (Murphy et al. 1996a, 1997; Ninan and Kulkarni 1999). These findings point out an intricate balance between the various subtypes of the dopamine receptor in cognitive functions, and it appears that both D_1- and D_2-like receptors may contribute to the memory deficit caused by FG-7142.

Dopamine D_1 and D_3 receptors are co-expressed in neurons in the nucleus accumbens and island of Calleja and in both regions were shown to produce synergistic actions (Barik and de Beaurepaire 1998; Schwartz et al. 1998). One may assume that concurrent stimulation of cortical D_1 and D_3 receptors—due to increased dopamine release by FG-7142—may result in similar synergism. By blocking the D_3 receptor component of this synergistic activation, D_3 antagonist ligands may indirectly diminish the consequence of excessive stimulation of dopamine D_1 receptors. In this way, they can contribute to restoration of the imbalance in prefronto-cortical dopaminergic activation and thus improve cognitive performance. Since the cortical dopamine level is elevated during FG-7142 amnesia a partial agonist may act functionally as an antagonist. This condition explains the similar efficacy of BP-897 to those of the full D_3 receptor antagonists.

Scopolamine, as a centrally active anticholinergic drug, is generally used to impair the cognitive functions in a wide variety of learning paradigms (Myhrer 2003). It is well documented that the neural basis of memory involves the interactions between the dopaminergic and cholinergic

neurotransmitter systems (Levin et al. 1990; Levin and Eisner 1994; Hersi et al. 1995; Umegaki et al. 2001). Evidence exists that supports a putative role of the D_3 receptor in the interaction with cholinergic mechanisms. The activation of striatal dopamine D_2/D_3 receptors by 7-OH-DPAT inhibited basal acetylcholine release (Sato et al. 1994), while in the study of Sigala et al. (1997) the moderately D_3 receptor-preferring antagonist nafadotride was found to inhibit the memory disruption elicited by scopolamine in a passive avoidance paradigm. Furthermore, according to a recent study (Lacroix et al. 2003), the systemic administration of the highly selective D_3 receptor antagonist SB-277011 (10 mg/kg i.p.) produced a significant increase in extracellular acetylcholine level in the medial prefrontal cortex. This finding is in good accordance with the considerable protective effect of SB-277011 against scopolamine amnesia obtained in our experiments and suggests that the elevation of prefrontal–cortical acetylcholine release may be one of the potential components involved in the memory-enhancing activity of SB-277011. Presumably, the other two D_3 antagonists tested—U-99194A and RGH-1756—might produce their learning improvement activity in scopolamine-treated rats by a similar stimulative effect on the cholinergic system. On the other hand, the D_3 partial agonist BP-897 failed to affect the scopolamine-induced learning deficit in our experiment, suggesting that the protection of learning disturbance induced by scopolamine requires full antagonism (i.e., complete blockade) on the D_3 receptor. It is also conceivable that during scopolamine amnesia, the endogenous dopamine tone remains low; thus, BP-897 acts more as an agonist.

As an alternative explanation to the dopamine D_3 receptor antagonism, one may assume that the molecules produced their antiamnesic effects via other receptor mechanisms. Although this possibility cannot be entirely excluded on the basis of the receptor profiles and the applied doses of the compounds, this notion is unlikely. Each compound has its strongest affinity to the D_3 receptor, usually followed by affinity to the D_2 receptor. However, on D_2 receptors all compounds are antagonists (Pilla et al. 1999; Reavill et al. 2000; Waters et al. 1993; Horváth et al. 2001), but D_2 antagonists impair rather than improve learning capabilities (see Myhrer 2003 for review). Furthermore, the compounds at the applied doses did not show the expected D_2 receptor-mediated effects (Reavill et al. 2000; Waters et al. 1993; Horváth et al. 2001). As far as other, non-dopaminergic receptors are concerned, (1) there is no common target of the compounds, and (2) any other potentially significant binding was found only in the same range as their D_2 affinity. SB277011 has low affinity to the 5-HT_{1D} and 5-HT_{2B} receptors (Reavill et al. 2000), U-99194A to muscarinic M_1 receptors (Audinot et al. 1998), BP-897 to alpha$_1$, alpha$_2$ and 5-HT_{1A} receptors (Pilla et al. 1999). RGH-1756 is an exception in that its 5-HT_{1A} affinity lies midway between its D_3 and D_2 binding (Kiss et al. 2000). The common feature among the compounds is their outstanding D_3 activity, which provides the most probable explanation for their cognition-enhancing characteristics. However, direct evidence for this hypothesis is currently unavailable.

In conclusion, all the functional antagonists of the dopamine D_3 receptor tested in our experiments possessed memory-improving properties, although to different degrees. This effect may be beneficial in those psychiatric diseases, such as schizophrenia, where patients show well-characterised cognitive dysfunction. Compounds that antagonise the dopamine D_3 receptor may therefore provide a promising therapy.

Acknowledgements The authors thank Mrs. Csilla Nagy and Mrs. Tünde Féth for their careful technical assistance.

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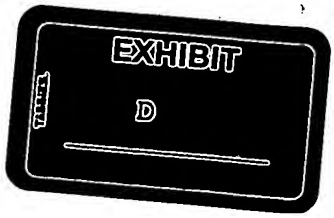

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U.S. Patent Application Serial No. 60/462,782
Inventor: Braje, et al
Filed: 04/14/2003

September 9, 2003

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